

Plant eco-physiological responses to multiple environmental and climate changes

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Plant eco-physiological responses to multiple environmental and climate changes



Ph. D. Thesis By
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Preface

This thesis on plant eco-physiological responses to multiple environmental and climate changes is the result of a three year ph.d project at Institute of Biology, University of Copenhagen and the Biosystems Department at RISØ-DTU. The studies have been interrupted by a two weeks maternity leave and 9 month research assistant position at the Biosystems department at RISØ-DTU. My supervisors Helge Ro-Poulsen and Teis N. Mikkelsen were always helpful with ideas, resources, and feedback when ever needed. In line with my supervisors I would like to express my gratitude to Anders Michelsen giving me extraordinary feedback all the way. I have been very fortunate to be a part of the physiological ecology research group that has been my scientific playground and room for many fruitful discussions. Thanks to Esben Vedel Nielsen, Gosha Sylvester, Niels Bruun, Karna Heinsen, Karin Larsen and Svend Danbæk for help with laboratory and IT work. In the CLIMAITE project group I have experienced a unique interdisciplinary teamwork of researchers in an open minded atmosphere, combined with ambitions and common research goals. This has been a platform for development, research and teamwork, and I have felt to be part of a research effort pushing insight far further than individually studies could have done. Thanks to all CLIMAITE members, the ph.d group and in particular thanks to project leader Claus Beier providing trust and resources into this ph.d project. CLIMAITE field work took place at the CLIMAITE research facility in Brandbjerg, Denmark and Svend Danbæk, Poul T. Sørensen and Preben Jørgensen is thanked for their timeless effort in keeping the site running. Fieldworkers Christel Barker and Niels Bruun are thanked for helping with measurement campaigns at Brandbjerg. Arctic field work took place in Zackenberg, NE Greenland and The Danish Polar Center (DPC) provided logistics and excellent conditions for research. Marie Frost Arndal and Kristine Boesgaard is thanked for their help in the field. Thanks to my family and friends for being patience, supportive and provide resources all the way. In particular my wife Katrine who has endured and supported me more than any could ask for.

Kristian Rost Albert, March 2009

'Set all things in their own peculiar place and know that order is the greatest grace'

John Dryden (1631-1700)

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Papers I-VIII

List of papers

This thesis is based on studies in the following papers, referred to by their Roman numerals in the introduction.

- I. Albert KR, Kongstad J, Schmidt IK, Ro-Poulsen H, Mikkelsen TN, Michelsen A, Beier C. Growth and eco-physiological responses of *Calluna vulgaris* and *Deschampsia flexuosa* to natural drought and warm autumn in a temperate heath. *Manuscript*.
- II. Albert KR, Ro-Poulsen H, Mikkelsen TN, Michelsen A, Beier C. Impact of elevated CO₂, warming and drought on photosynthesis of *Deschampsia flexuosa* in a temperate heath ecosystem. *Manuscript*.
- III. Albert KR, Ro-Poulsen H, Mikkelsen TN, Michelsen A, Beier C. Prolonged growing season and complex water relations in interactions of CO₂, warming and drought on *Calluna vulgaris* in a temperate heath. *Manuscript*.
- IV. Albert KR, Ro-Poulsen H, Mikkelsen TN, Michelsen A. Photosynthetic performance differences in response to elevated CO₂, warming and drought: Comparing an opportunist grass to an evergreen dwarf shrub in a temperate heath ecosystem. *Manuscript*.
- V. Albert KR, Ro-Poulsen H, Mikkelsen TN (2008) Ambient UV-B radiation decreases photosynthesis in high arctic *Vaccinium uliginosum*. *Physiologia Plantarum* 133: 199-210.
- VI. Albert KR, Rinnan R, Ro-Poulsen H, Mikkelsen TN, Håkansson KB, Arndal MF, Michelsen A (2008) Solar Ultraviolet-B Radiation (UV-B) at Zackenberg: The Impact on Higher Plants and Soil Microbial Communities. *In*: Meltofte H, Christensen T, Elberling B, Forchhammer M, Rasch M. (Eds.). Dynamics of a High Arctic Ecosystem, *Advances in Ecological Research* 40: 421-440. Elsevier.
- VII. Albert KR, Mikkelsen TN, Ro-Poulsen H, Arndal MF, Michelsen A. Ambient UV-B doses decreases PSII performance and net photosynthesis under natural irradiance in high arctic *Salix arctica* heath. Submitted to *Plant, Cell and Environment*.
- VIII. Albert KR, Arndal MF, Boesgaard K, Mikkelsen TN, Michelsen A, Håkansson KB, Schmidt NM, Ro-Poulsen H. Ambient UV-radiation decreases PSII performance in spite of increased screening ability via UV-absorbing compounds in *Salix arctica* and *Vaccinium uliginosum* during six years of UV-exclusion in high arctic heaths. Submitted to *Environmental and Experimental Biology*.

Summary

The current global changes of temperature, precipitation, atmospheric CO₂ and UV-B radiation impact in concert ecosystems and processes in an unpredictable way. Therefore multifactor experimentation is needed to unravel the variability in strength of these drivers, whether the factors act additively or synergistically and to establish cause-effect relations between ecosystem processes.

This thesis deals with heath plant responses to global change factors (the CLIMAITE project). In a Danish temperate heath ecosystem elevated CO₂, experimental summer drought, and passive nighttime warming was applied in all combinations (based on the scenario for Denmark anno 2075) and the responses after one year of treatment were investigated through a growing season in Hairgrass (*Deschampsia flexuosa*) and Heather (*Calluna vulgaris*). In a high arctic heath ecosystem situated in NE-Greenland UV-B exclusion experiments were conducted on *Salix arctica* and *Vaccinium uliginosum* during six years. Responses of photosynthesis performance were characterized on the leaf scale by means of leaf gas-exchange (A/C_i curves), chlorophyll-a fluorescence, leaf nitrogen, carbon and $\delta^{13}\text{C}$ and secondary compounds.

The main findings were 1) The different growth strategies of the evergreen *Calluna* versus the opportunistic bi-phasic *Deschampsia* affects the photosynthesis response to drought and autumn warming (Paper I); 2) Elevated CO₂ and warming synergistically increase photosynthesis in spring and autumn (Paper II and III); 3) Summer drought decreased photosynthesis in both species, but where *Calluna* maintained photosynthetic metabolism then major proportion of grass leaves wilted down (Paper I); 4) Elevated CO₂ did not decrease stomatal conductance, but the treatments affected soil water content positively, pointing to the complex water relations when plants of contrasting growth strategy co-occur (Paper III and IV); 5) Water availability affected the magnitude of photosynthesis to a higher degree than warming and elevated CO₂ (Paper II and III); 6) Photosynthetic capacity were closely linked to growth strategy and rewetting stimulation were closely related to high nitrogen leaf content (Paper IV); 7) Responses to elevated CO₂, drought and

warming could not be deduced from single factor experiments (Paper II and III); 8) Ambient UV-B decreased PSII performance despite stimulation of UV-B absorbing compounds in high arctic plants in both short and long term (Paper V, VII, VIII); 9) Ambient UV-B decreased net photosynthesis via effects on PSII performance in combination with effects on Calvin Cycle (Paper V, VII); 10) Current UV-B level is an important factor affecting high arctic plants, particularly in years with spring advancement induced by warming (Paper VI).

In conclusion, the results in this thesis suggest the responses of temperate heath plant photosynthesis to be imitatively linked with plant growth strategy and water relations, and also that the responses of photosynthesis to the important drivers often interacted. Current UV-B levels decrease productivity in high arctic heath plants, and advanced spring in response to warming may lead to further decrease while other climatic changes as elevated CO₂ may negate this. Stimulated productivity of temperate heath plants is likely under the climatic conditions predicted to be prevailing in Denmark anno 2075.

Sammenfatning (Danish summary)

De nuværende globale ændringer af temperatur, nedbør, forhøjet niveau af kuldioxid koncentration og ultraviolet stråling er faktorer som tilsammen påvirker et økosystem på en ikke altid forudsigelig måde. Derfor er multi-faktor eksperimenter essentielle for at belyse disse faktorerers varierende styrke, om faktorerens påvirkning er additiv eller synergistisk, og for at fastslå sammenhængen mellem årsag og virkning i det komplicerede samspil mellem de forskellige processer i økosystemet.

I denne ph.d rapport undersøgte hedeplanters respons til globale klimaændringer i CLIMAITE projektet. I et dansk hede økosystem blev forhøjet kuldioxid kombineret med forlænget sommertørke og passiv natopvarmning (baseret på et realistisk klimascenarie for Danmark anno 2075). Efter efter et års behandling blev påvirkningen af planternes kulstofoptag og fotosyntese karakteristika undersøgt på Hedelyng (*Calluna vulgaris*) og Bølget Bunke (*Deschampsia flexouosa*). Desuden gennemførtes i et hedeøkosystem i høj-arktis over 6 år en række UV-B eksklusions forsøg på Arktisk Pil (*Salix arctica*) og Mosebølle (*Vaccinium uliginosum*). Fotosyntesens respons blev karakteriseret på bladniveau ved hjælp af gasudveksling af CO₂ og H₂O (CO₂-respons kurver), klorofyl fluorescens og målinger af bladenes indhold af nitrogen, kulstof, sekundærstoffer og $\delta^{13}\text{C}$.

Hovedresultaterne er at 1) Vækst-strategierne i den stedsegrønne dværgbusk Hedelyng og den opportunistiske græs Bølget Bunke påvirkede fotosyntesens størrelse og karakteristika under naturlig tørke og i et usædvanligt varmt efterår (Manuskript I); 2) Opvarmning og det forhøjede niveau af kuldioxid øgede synergistisk planternes kulstof optag i forår og efteråret (Manuskript II og III); 3) Sommertørke nedsatte planternes kulstofoptag, men hvor Hedelyng opretholdt den fysiologiske aktivitet i sine skud så visnede en stor del af Bølget Bunke bladene (Manuskript I); 4) Forhøjet niveau af kuldioxid nedsatte ikke bladenes åbningsgrad, men det bevarede mere af vand i jorden. Dette indikerer komplekse vand-relationer, når planter med forskellige vækst-strategier

sameksisterer (Manuskript III og IV); 5) Planternes vandtilgængelighed påvirkede fotosynteseratens størrelse i højere grad end opvarmning og forhøjet niveau af kuldioxid (Manuskript II og III); 6) Den maksimale fotosyntetiske kapacitet var tæt relateret til planternes vækstrategi og stimuleringen af den maksimale fotosyntetiske kapacitet efter nedbør var tæt relateret til et højt næringsstofniveau i bladene (Manuskript IV); 7) Planternes forøgede kulstofoptag og fotosyntesens karakteristika i den kombinerede behandling af forhøjet kuldioxid, tørke og opvarmning (som bedst repræsenterer Danmark anno 2075) kunne ikke forudsiges ud fra enkeltfaktor forsøgene (Manuskript II, III og IV); 8) Både på kort og på langt sigt var planternes lysprocessering og elektron transport negativt påvirket af det nuværende niveau af ultraviolet stråling på trods af at flere beskyttende UV-B absorberende stoffer forekom i bladene (Manuskript V, VII, VIII); 9) Ultraviolet stråling nedsatte fotosynteseraten via en kombineret negativ effekt på Fotosystem II og i Calvin Cyclus (Manuskript V, VII); 10) Ultraviolet stråling er en vigtig faktor som påvirker højarktiske hedeplanter, især når et tidligt forår som følge af opvarmning øger UV-B dosis.

Disse resultaterne fra CLIMATE-projektet påpeger en tæt sammenhæng mellem planternes vækst-strategi, vandforholdene og fotosyntesens respons. Desuden var der hyppige interaktioner mellem det forøgede kuldioxid niveau, opvarmning og tørke på planternes kulstofoptag. I den behandling som bedst repræsenterer Danmark i fremtiden sås et forhøjet kulstofoptag, især i forår og efterår.

1. Background and objectives

1.1 *Climate change and multi-factor experiments*

The climate is changing and the anthropogenic forcing have been thoroughly documented (IPCC 2001, 2007). The level of atmospheric CO₂ have increased from 270 ppm in the 18th century to current values around 380 ppm and is expected to increase up to a range around 700 ppm in this century (IPCC 2001). Warming will be more pronounced during nighttime and temperature increases in the range 1.4-5.8°C is expected during the next 100 years (IPCC 2001). Precipitation changes with prolonged summer droughts, heavy precipitation events and higher frequency of extremes are also expected (IPCC 2007). Regional modelling of precipitation for Europe shows an increased precipitation in Denmark during autumn, winter and spring. However a decrease during summer results in longer drought periods, but the incidence of severe precipitation could increase (Christensen and Christensen 2003).

This has attracted focus on the ecosystem responses to climatic changes and also on the ecosystem feedbacks to climate, and large effort are invested in understanding these concerted and complex processes (Callaghan *et al.* 2004a, 2005, Heimann and Reichstein 2008, Rustad 2006, 2008). Since all environmental changes will occur concurrently and plants and ecosystems are affected interactively it is important to manipulate all environmental changes at the same time. Hence, a tool to improve understanding is the analysis of responses in multi-factor experimental manipulation studies (Beier *et al.* 2004, Classens and Langley 2005, Heimann and Reichstein 2008, Norby and Lou 2004). Multifactor experiments are logistically and economically demanding to run, but they have the advantage to provide observations with a close coupling of cause-effect relationship and they include both direct and indirect effects between the manipulated drivers (see e.g. Rustad 2008). Despite substantial knowledge of impact of single factors as e.g. elevated CO₂ (e.g. Ainsworth and Long 2005, Ainsworth and Rogers 2007, Körner 2000, Drake *et al.* 1997), drought (e.g.

Chaves 1991, Schulze 1986, Yordanov *et al.* 2000) and warming (e.g. Lou 2007, Penuelas and Filella 2001), then multi-factor studies have the potential to reveal gaps in our knowledge as the interactions between manipulated drivers may act additively or synergistically and thereby yield surprising results (Henry *et al.* 2005, Lou *et al.* 2008, Norby *et al.* 2007, Shaw *et al.* 2002). By the expression of 'mucking through multifactor experiments' Dermody (2006) draw attention to the challenge to analyze and understand processes and responses across scale in ecosystem manipulations. When initial results are followed by re-interpretations and re-experimentations then the multi-factorial approach have the advantage to facilitate in depth investigations of the causal mechanisms involved, gain new insight and push understanding further forward (Heimann and Reichstein 2008, Rustad 2008). Preferentially such studies can be a part of an integrated unified multidisciplinary approach to evaluate ecosystem change (Classen and Langley 2005, Norby and Lou 2004, Rustad 2006) including non-destructive ecosystem level observations and the integration of these results into ecosystem modelling (Heimann and Reichstein 2008). With time the concerted impact of the manipulations on the ecosystem processes and pools likely approach and a steady state and therefore long term maintenance of experiments are of special importance (Ainsworth and Long 2005, Heimann and Reichstein 2008, Shaver *et al.* 2000). However, even though long term experimental manipulations provide insights on ecosystem response on annual to decadal time scale, then these types of manipulations do not simulate the gradual change in climate that occurs on the century to millennium scale. Therefore this approach can be combined with studies of ecosystem dynamics along natural climatic and environmental gradients at millennium time scale to effectively put bounds on what is likely to occur on the century time scale (Callaghan *et al.* 1999, Rustad *et al.* 2001).

1.2 Warming

Temperature is a key factor that regulates many terrestrial biogeochemical processes such as plant productivity (Sage and Kubien 2007, Warren-Wilson 1957), plant nutrient uptake (Bassirad *et al.* 2000), root dynamics (Gill and Jackson

2000, Pregitzer *et al.* 2000), nutrient mineralization (MacDonald *et al.* 1995, Malhi *et al.* 1990), soil respiration (Raich and Schlesinger 1992) and litter decomposition (Hobbie 1996, Meentemeyer 1978) and this makes it difficult to predict the overall ecosystem responses to changes in temperature (Shaver *et al.* 2000). Providing a conceptual framework for analyzing effects of warming on ecosystem processes, Shaver *et al.* (2000) points to both direct and indirect effects adding to the complex impact of this driver.

Experimental warming with a range of approaches has been demonstrated to increase measures of productivity (Arft *et al.* 1999, Dukes *et al.* 2005, Rustad *et al.* 2001, Shaw *et al.* 2002). E.g. passive night-time warming enhanced the carbon fixation at least in the short term, with strongest responses on sites with low aridity (Penuelas *et al.* 2007). However the underlying causal mechanisms are diverse e.g. warming can increase the length of the growing season (Cleland *et al.* 2006, Menzel and Fabian 1999, Piao *et al.* 2007, Walter *et al.* 2002, White *et al.* 1999), change plant phenology (Harte and Shaw 1995, Menzel *et al.* 2006, Wan *et al.* 2005), increase nutrient mineralization and availability (Rustad *et al.* 2001), increase soil respiration (Piao *et al.* 2007, Rustad *et al.* 2001), reduce soil water content (Morgan *et al.* 2004, Wan *et al.* 2005, Volk *et al.* 2000) and shift species composition and community structure (Shaver *et al.* 2000, Wan *et al.* 2005). Direct effects could provide closer to optimal growth temperature and increase photosynthetic rates (Sage and Kubien 2007). Respiration response to temperature may either increase respiration or some acclimation to temperature may occur and possibly counteract the expected respiration increase (Atkin and Tjoelker 2003). Nighttime warming can increase night time respiration and stimulate sink strength and net photosynthesis during daytime (Turnbull *et al.* 2002, 2004). Indeed the influence of respiration on carbon balance can be substantial (Penuelas *et al.* 2007, Piao *et al.* 2007, Schindlbacher *et al.* 2009, Valentini *et al.* 2000). Indirect effects of warming via soil water availability and via nutrients in particular have been argued to be more important on photosynthesis than direct effects (Körner 2000, Lou 2007, Morgan *et al.* 2004, Shaver *et al.* 2000).

1.3 Water relations

Drought and heat wave events can have profound negative impact on productivity (Ciais *et al.* 2005, Heisler and Weltzin 2006, Penuelas *et al.* 2007, Rustad 2008). The variability in rainfall has the potential to change CO₂ uptake in grassland and productivity can be more responsive to soil moisture variability than to mean SWC (Knapp *et al.* 2002, Volk *et al.* 2000). Precipitation experiments on the ecosystem scale have been performed along a European gradient (Penuelas *et al.* 2004, Beier *et al.* 2004, Emmett *et al.* 2004), showing negative effects on productivity in response to drought, but the magnitude in response depend much on differences between sites, years, and species (Penuleas *et al.* 2007). For an overview of other ecosystem experiments see Rustad (2008). Besides the amount and timing of precipitation, plant community changes can mediate the productivity responses via differences in plant physiology of the dominant species (Heisler and Weltzin 2006, Knapp *et al.* 2001).

The water stress induced limitation on plant growth is mainly caused by reductions in plant carbon balance, i.e. the balance between photosynthesis and respiration (e.g. Flexas *et al.* 2006a). The response of photosynthesis to water stress has received considerable attention in the past, and there has been a long-standing controversy regarding what is the primary limitation on photosynthesis, stomatal closure or metabolic impairment (Boyer 1976, Chaves 1991, Cornic and Massacci 1996, Jones 1973, Lawlor 1995, Lawlor 2002). Non-stomatal limitations were mostly attributed to the presence of heterogeneous stomatal closure during drought (Downton *et al.* 1988, Terashima *et al.* 1988). However, evidence has been accumulating showing that photophosphorylation (Havaux *et al.* 1987, Meyer and de Kouchkovsky 1992), RuBP regeneration (Giménez *et al.* 1992, Gunasekera and Berkowitz 1993) and Rubisco activity (Castrillo and Calcagno 1989, Medrano *et al.* 1997) are impaired under drought. E.g. it was found that impaired photophosphorylation and ATP synthesis was the main factor limiting photosynthesis in sunflower, even under mild drought (Tezara *et al.* 1999). In recent years, efforts have been made to generalize the responses to water stress of photosynthetic parameters in higher plants (Chaves *et al.* 2003, Flexas and

Medrano 2002, Lawlor and Cornic 2002). As a result, there is now some consensus that diffusion limitations on photosynthesis predominate under most water-stress situations. These limitations involve not only stomatal closure, but also decreased mesophyll conductance to CO₂, an important but sometimes neglected process (Flexas *et al.* 2002, Ennahli and Earl 2005, Flexas *et al.* 2007). Regardless of the species analyzed a general failure of metabolism occurs only when daily maximum stomatal conductance drops below 0.1 mol H₂O m²/s (Flexas *et al.* 2004, Grassi and Magnani, 2005). As discussed in (Flexas *et al.* 2006) stomatal and mesophyll conductance dominate the photosynthesis limitation when daily maximal stomatal conductance is above 0.05-0.10 mol H₂O m²/s and general metabolic impairments occurs below that threshold. On the other hand, the carbon balance of a plant enduring a water-stress period may depend as much on the rate and degree of photosynthetic recovery as on the rate and degree of photosynthetic decline during water depletion. While many studies have addressed different aspects of photosynthetic limitations during water-stress imposition, analyses of the photosynthetic limitations during photosynthetic recovery after water stress are scarce (Galmes *et al.* 2007, Miyashita *et al.* 2005, Flexas *et al.* 2006a). An early study by Kirschbaum (1987,1988) suggested that photosynthesis during recovery was co-limited by incomplete stomatal opening and a metabolic component. Recently, Ennahli and Earl (2005) have suggested that recovery of photosynthetic biochemistry was the most important limitation for photosynthetic recovery in cotton plants subjected to severe water stress. The incorporation of understanding of such metabolic processes and not only stomatal conductance have been stressed to be of particular importance when modeling of productivity in response to precipitation has to be made (Gerten *et al.* 2008, Reichstein *et al.* 2002, Lou *et al.* 2008).

The plant canopy is the interface for the physiological and physical processes that control energy and water exchange between the atmosphere and the terrestrial biosphere. Besides from stomatal conductance regulations the size of the canopy are often closely linked to soil moisture content (Murthy *et al.* 2005). Limited soil moisture often leads to decreased maximum LAI and earlier

senescence (Harper *et al.* 2005, Wand *et al.* 1999). Although evapo-transpiration may be reduced, such declines in LAI may actually exacerbate soil moisture losses, because of increased heat load and evaporation at the soil surface (Obrist *et al.* 2003, Wan *et al.* 2002). The responses of LAI will in turn affect soil moisture contents e.g. higher LAI may increase the surface for evapo-transpiration (Phillips *et al.* 2006). Alternatively, loss of soil moisture in warm and dry conditions may be slowed if decline in LAI also occur (Wan *et al.* 2002, Zavaleta *et al.* 2003). Therefore a close coupling between plant growth pattern and the resulting LAI influence soil moisture contents and improved understanding is crucial to model future ecosystem productivity (Cowling and Field 2003, Ewert 2004, Filella *et al.* 2004).

1.4 Atmospheric elevated CO₂

Effects of increasing CO₂ has received pronounced interest and has been studied during the last 15-20 years in open top chambers and with the FACE technique in several ecosystems (Ainsworth and Long, Drake *et al.* 1997, Delucia *et al.* 2005, Kimball *et al.* 2007, Norby *et al.* 2005, Nowak *et al.* 2004, Morgan *et al.* 2004). Elevated CO₂ have been demonstrated to increase ecosystem carbon input and the fate of the extra carbon can be numerous e.g. respiration in soil, plants and other organisms, trapped in pools of variable size e.g. soil organic matter, dissolved organic matter, microbial and other living biomass, and therefore does not necessarily translate into biomass production (Körner 2003, 2006, Nowak *et al.* 2004). Moderate stimulated plant biomass in elevated CO₂ is expected in natural ecosystems with natural nutrient cycle, fully developed leaf area index and fine root renewal (Körner 2006) such as steady state grassland, where effects are argued to be water related, and in closed forest stands where the initial stimulations decline with time (Körner 2006, Morgan *et al.* 2004).

Elevated CO₂ affects plant eco-physiology in several ways (see 2.3 for description of parameters). Predominantly elevated CO₂ decreases the stomatal conductance, stimulates the light saturated net photosynthesis and increases the water use efficiency (Ainsworth and Rogers 2007, Ainsworth and Long 2005,

Curtis 1996, Curtis and Wang 1998, Medlyn *et al.* 2001). Acclimation to elevated CO₂ often decreases the photosynthetic capacity via down-regulation of primarily the maximum carboxylation rate, V_{cmax} , and to some degree also maximum rate of electron transport, J_{max} (Ainsworth and Rogers 2007, Ainsworth and Long 2005, Curtis 1996, Curtis and Wang 1998). In a meta-analysis, grasses showed a reduction in V_{cmax} approximately double the reduction in J_{max} (Ainsworth and Rogers 2007). As CO₂ rises, control by the capacity for light saturated photosynthesis by Rubisco (V_{cmax}) decreases and control by the capacity for RubP regeneration (J_{max}) increases (Long and Drake 1992, Long *et al.* 2006a). Therefore the excess capacity for carboxylation are being reduced through reduction of Rubisco content and redistribution of the excess N invested in Rubisco if resources invested can be used beneficial elsewhere (Drake *et al.* 1997, Parry *et al.* 2003). This response lead to the decrease in leaf nitrogen content and increased leaf C/N ratio in many studies as summarized by e.g. Ainsworth and Long (2005) or Ainsworth and Rogers (2007). The magnitude of the response of photosynthesis to elevated CO₂ is modulated by nitrogen supply and by constrains of the capacity to utilize the photosynthates (Ainsworth and Rogers 2007). In the long term the nutrient cycling may limit the stimulation of carbon binding in plant production (Finzi *et al.* 2002, Reich *et al.* 2006). However, reduced sink capacity may result from other limiting processes than nutrient supply (Rogers *et al.* 1998), temperature (Ainsworth *et al.* 2003), developmental changes (Rogers *et al.* 2004, Bernacchi *et al.* 2005, Rogers and Ainsworth 2006), or other restricting environmental variables that can modulate the well characterized primary responses of elevated CO₂ on stomatal conductance and net photosynthesis (Ainsworth and Rogers 2007). Importance of the plant water relations via 'CO₂ water savings' has been particularly stressed in grasslands and forests exposed to elevated CO₂ (Volk *et al.* 2000, Morgan *et al.* 2004, Körner 2000, Leuzinger and Körner 2008). As discussed above elevated CO₂ improves the water use efficiency via decreased stomatal conductance reducing the water consumption (Morison and Gifford 1984, Medlyn *et al.* 2001). This will lead to decreased soil moisture depletion if the vapour pressure gradient across the leaf boundary layer stays the same and if the

stomatal response is not compensated by increased leaf area index (Kelliher *et al.* 1993). Therefore net photosynthesis at progressively decreasing water availability has been hypothesized to be sustainable for a longer period in elevated CO₂ (Drake *et al.* 1997, Wall *et al.* 2001, Robredo *et al.* 2007), and in habitats with periodic drought this could extend the period of active growth. There is clear evidence that soils under grassland exposed to elevated CO₂ maintain higher moisture (Fredeen *et al.* 1997, Niklaus *et al.* 1998) and thus water status may strongly co-determine plant biomass responses to CO₂ enrichment (Acock and Allen 1985, Gifford 1992, Koch and Mooney 1996, Field *et al.* 1997). Attempts to structure eco-physiological responses from plant functional types have been made (Arft *et al.* 1999, Chapin *et al.* 1996, Walker *et al.* 2006). Some studies find dwarf shrubs less responsive to environmental perturbations and the opportunistic grasses to be more responsive (Aerts 1995, Chapin and Shaver 1996, Graglia *et al.* 2001, Michelsen *et al.* 1999). Consequently, species that differ in their response to environment and their effects on ecosystem processes, in contrast to the buffering capacity provided by ecologically similar species, can make ecosystems more vulnerable to change (Chapin *et al.* 1997). Therefore characterisation of individual species is of importance.

1.5 Ambient UV-B

The stratospheric ozone layer reduces the solar UV-B radiation (280-315 nm) reaching the earth surface (Kerr 1993, McKenzie *et al.* 1999), but stratospheric ozone depletion increases the UV-B radiation, especially in the Arctic and Antarctic (Madronich *et al.* 1998, Webb 1997). Currently, the UV-B irradiance level in the arctic region is considered to be near its maximum, and the ozone column is estimated to recover towards the middle of the century (Shindell *et al.* 1998, Taalas *et al.* 2000). However, rate of ozone recovery is uncertain in the northern hemisphere (WMO 2003) as stratospheric cooling by greenhouse gasses is increasing the formation of polar stratospheric clouds. These are sites of ozone-destroying reactions and elevated UV-B levels may persist over the Arctic for several decades (WMO 2003, Weatherhead *et al.* 2005). Increased cloudiness

decreases the amount of UV-B radiation reaching the ground (Madronich *et al.* 1998), but the predictions of future cloud cover and cloud types are uncertain (Weatherhead *et al.* 2005). The biological impact of a persistence of the current level of UV-B radiation is particularly important when pronounced warming causes earlier snow melt and start of growing season prolongs the exposure of the vegetation to the higher spring UV-B fluxes.

High-arctic plants are on the limit of their distribution in an extreme environment with a short growing season, low temperatures and often nutrient limitation. Therefore, acclimation is of special importance especially when the plants face environmental changes, such as increased UV-B radiation (Caldwell *et al.* 1980, Robberecht *et al.* 1980), which can cause additional stress under ambient conditions (Bredahl *et al.* 2004, Albert *et al.* 2005). In addition, because the vegetation in the Arctic is evolutionary adapted to low UV-B levels (Caldwell *et al.* 1994, Robberecht *et al.* 1980), the potential impact on the vegetation is expected to be pronounced (Björn *et al.* 1999, Paul, 2001). This leads to the hypothesis that arctic plants are negatively influenced by the current UV-B levels. Thus, if the present UV radiation affects the vegetation significantly, then reduction of the irradiance load would improve the photosynthetic performance of the plants.

Studies on ecological responses to UV-B radiation in the Arctic are a research area of current interest (Callaghan *et al.* 2004a, 2005), with widely variable and often limited responses of plants to perturbations (Callaghan *et al.* 2004b, Phoenix *et al.* 2001, 2002, Rozema *et al.* 2006). Knowledge of polar ecosystem responses to UV-irradiance is the result of field experiments with various experimental approaches: UV-radiation has been elevated by various lamp setups (e.g. Björn *et al.* 1999, Gwynn-Jones *et al.* 1997, Johanson *et al.* 1995a, 1995b, Phoenix *et al.* 2001), transplants have been set up along latitudinal gradients (Lehrner *et al.* 2001), or UV radiation has been reduced by means of filters (Albert *et al.* 2005, Phoenix *et al.* 2002, Robson *et al.* 2003, Xiong and Day 2001). In principle, the studies supplementing UV-B relate closely to scenarios with future increased UV-B levels, whereas experimental UV-reduction relates to the impacts of the current level of solar UV-radiation. The UV-exclusion experiments

by means of filters are attractive in several ways. They are simple and do not require electrical power or any special technical maintenance, which is an advantage in remote areas. Further, differences in spectral ratios, which are a problem in the UV supplementation experiments (Caldwell and Flint 1994), can be avoided. However, reduction of the UV-B irradiance by 60% or more implies a higher relative change in the UV-B load than is predicted to take place in nature. Anyhow, the clear advantage to emphasize here is that the interpretation of results from UV-exclusion experiments directly relates to the impact of current level of UV-radiation, and that the exposure includes the variability during the growing season and from year to year.

In general the UV-B radiation have been reported to induce increased production of UV-B absorbing compounds (Day *et al.* 2001, Searles *et al.* 2001a), increased production of berries (Gwynn-Jones *et al.* 1997), microbial community changes (Riinnan *et al.* 2005), alters the below ground processes (Johnson *et al.* 2002), decrease plant growth (Ballaré *et al.* 2001, Day *et al.* 1999, Rousseaux *et al.* 1999, Ruhland and Day 2000), DNA damage (Robson *et al.* 2003, Rousseaux *et al.* 1999, Ruhland *et al.* 2005, Xiong and Day 2001), slowed the rate of litter decomposition, altered microbial soil biomass (Johnson *et al.* 2002) and reduced cyanobacterial nitrogen fixation (Solheim *et al.* 2002), but did not change plant cover or DNA damage (Rozema *et al.* 2005 and references herein). Further, after seven years of UV-B supplementation no significant effects were detected on plant cover, plant density, leaf weight, leaf area, reproductive parameters, leaf UV-B absorbance and content of total phenolics in plants on arctic Svalbard (Rozema *et al.* 2006). Based on the findings in above field studies and several others the prevalent view is that arctic plants are more or less tolerant to enhanced UV-B in the long term (Callaghan *et al.* 2004b, Phoenix *et al.* 2001, Rozema *et al.* 2006). Moreover, effects of other climatic changes such as elevated atmospheric CO₂, temperature or changes in precipitation may overwhelm the detrimental effects of enhanced UV-B in the sub arctic (Haapala *et al.* 2009, Phoenix *et al.* 2001, Phoenix & Lee 2004). However, little attention has been paid to the photosynthetic

performance in natural ecosystems in relation to ambient solar UV-B radiation in the Arctic (Albert *et al.* 2008b).

Warming advances spring substantially in some years in the high arctic (Høye *et al.* 2007). This change in snow-cover and length of the growing season may impact the UV-B exposure dose of the vegetation. Moreover, vegetation located in the snow-free patches will receive greatly increased UV-B dose due to irradiance reflected from the surrounding snow (Gröebner *et al.* 2000, Jokela *et al.* 1993).

1.6 Objective of this thesis

The objective of this Ph.d. project was to investigate the photosynthetic performance of heath plants in response to several important single factor environmental changes, their combinations and possible interaction effects. The following questions are treated in the eight included papers.

- How do differences in growth form and strategy affect the photosynthetic performance during the growing season? (Paper I)
- How do differences in growth form and strategy affect the photosynthetic capacity in response to elevated CO₂, warming and summer drought (Paper IV)
- How does advancement of the spring and extension of the growing season in the autumn affect photosynthetic performance in response to warming? (Paper II, III)
- How does water shortage caused by natural and experimental drought affect photosynthetic performance? (Paper II, III)
- How does elevated CO₂ affect the photosynthetic performance and water relations? (Paper II, III)
- Does elevated CO₂ and warming in combination increase plant carbon uptake and what are the consequences for the water relations? (Paper II, III)

- Does elevated CO₂ mitigate the negative impacts of decreased water availability in the response to natural and experimental drought? (Paper II, III)
- How does warming and experimental drought in combination affect the photosynthetic performance and water relations? (Paper II, III)
- Is ambient UV-B radiation affecting photosynthetic performance of high arctic heath plants? (Paper V, VI)
- Are high arctic plants acclimating to changes in ambient UV-B radiation in the long term? (Paper VII)
- Are variations in ambient irradiance of PAR and UV-B doses affecting photosynthetic performance of high arctic heath plants? (Paper VIII)

2. Approach and methodology

2.1 Temperate heath climatic change: The CLIMAITE Project

To study the effects on photosynthesis and a range of other important ecosystem processes a multifactor experiment with elevated CO₂, warming and drought were initiated October 2005 (CLIMAITE, Mikkelsen *et al.* 2008). In this project different work packages each characterize various components and processes e.g. vegetation composition and structure, phenology and flowering, biomass distribution above and below ground, response studies of enchytraerides and nematodes, root dynamics, nutrients, and leaf and ecosystem gas-exchange of CO₂, H₂O, N₂O and CH₄. Particularly focus has been on the carbon and nutrient cycles where the project is now in a phase of beginning synthesis. This is accomplished with the use of a comprehensive ecosystem model dealing with these processes and responses across scales in the ecosystem. The experimental manipulations chosen were adjusted to follow a scenario for Denmark in 2075 from the Danish Metrological Institute. It predicts a temperature raise increase in the order of 2°C, a CO₂ concentration above 650 ppm and long periods with summer drought. The experimental site is a dry heath land ecosystem on sandy soil in

North Zealand, Denmark, dominated by the evergreen dwarf shrub Heather (*Calluna vulgaris* L.) and Hairgrass (*Deschampsia flexuosa* L.). The plant cover was subjected to the following experimental treatments: Un-manipulated control (A), elevated CO₂ (CO₂), passive nighttime warming (T), summer drought (D), and all combinations (TD, TCO₂, DCO₂, TDCO₂) replicated in six blocks in a split-plot design. Each block consisted of two 8m diameter octagons of each divided in four plots. In one octagon CO₂ was elevated to 510 ppm during daylight hours with the FACE technique and in the other octagon CO₂ level was ambient. In each octagon automatized infrared reflective curtains covered one half during night preserving a larger proportion of the daily incoming radiation energy, which increased the night air temperature with 1.4°C in average. The plots perpendicular to the IR-curtains was periodically covered with a rain-excluding curtain which automatically was activated by rain, thus creating an artificial drought period (Mikkelsen *et al.* 2008). This split-plot design allowed statistical power as the number of replicates (n=6) is fairly high and to a high degree cover the natural variation at the site. With the block design a major part of the site heterogeneity could be ascribed to random variation in the statistical analysis. Comprehensive characterization of the site prior to onset of manipulations allow for some exclusion of these initial conditions when incorporation of co-variables (biotic and non-biotic) in the statistical analysis was made. Intensive climatic measurements were recorded e.g. in each experimental plot the temperature was measured in 2 and 5 cm depth and the soil moisture in 0-20 and 0-60 cm depth. Two climatic stations recorded precipitation, air temperature, photosynthetically active radiation, wind direction and relative humidity (Mikkelsen *et al.* 2008). The CO₂ and warming treatments were initiated 3rd October 2005. The drought treatment started 3rd July 2006 and was continued for 5 weeks until 4th August when soil water content reached c. 5 % water content in the top 20 cm of the soil. For further description of site and experimental setup, see Mikkelsen *et al.* (2008). Initial leaf gas-exchange measurements were performed in 2005 and intensified just before and after the start of the manipulations. The phenology of the *Calluna* shoots vary during season and also the needle shaped leaves of *Deschampsia* forced development of a new

methodological approach to secure reproducible measurements. Before each campaign *Calluna* shoots were therefore gently flattened between nylon strings positioning the shoots in a comparable light environment and enabling fast access for measurements. *Deschampsia* leaves were laid side by side without overlaps to secure a homogeneous leaf surface and fixed with hair pins just before measurements. Then light and CO₂ response curves were conducted within a window of 3-7 days for each species followed by harvest to determine leaf area, leaf carbon, leaf nitrogen and leaf $\delta^{13}\text{C}$ at a monthly basis from May to October 2006. Measurements of the plant water potential were conducted on the woody branches of *Calluna* July to October 2006.

2.2 High arctic heath UV-B exclusion studies

In high arctic Zackenberg effect studies of ambient UV-radiation reduction were performed from 2001 and onwards, and some of the manipulations are still maintained. Basically the rationale behind the experimentation is that if UV-radiation affects plant photosynthetic performance negatively then removal of a high proportion of UV-radiation leads to a measurable stress release. The experimental setup applied to various sites with different plant species composition where the responses of photosynthetic performance parameters on *Betula nana*, *Vaccinium uliginosum* and *Salix arctica* are being investigated. The four main treatments comprised open control plots without any filters, UV transparent filter controls, filters reducing UV-B (280–315 nm) and filters reducing both UV-B and UV-A (315–400 nm). These treatments exposed the vegetation to approximately 100%, 91%, 39% and 17% of ambient UV-B radiation and 100%, 97%, 90%, and 91% of the photosynthetically active radiation (PAR), respectively (Bredahl *et al.* 2004). Filters also slightly changed the microclimate when compared to the open control. Three succeeding years with measurements during the growing season revealed that the filters increased the mean soil temperature up to 0.6°C, but this did not affect soil humidity. No significant mean air temperature difference was observed within the filter treatments or between the filter treatments and the open control. Between the filter treatments no microclimatic differences were found.

Hereafter only periodically soil temperature and humidity measurements were done. To study the impact of variation in ambient irradiance doses the exposure of the sites dominated by *Salix arctica* and *Vaccinium uliginosum* has been varied and supplemented with leaf angle control on *Salix arctica*. Direct effects on leaf gas-exchange have been limited to *Salix arctica* as the leaf size of *Betula nana* and *Vaccinium uliginosum* is too small. However, by inserting chamber bases the leaf gas-exchange of whole canopies of *Vaccinium uliginosum* was measured. Besides the regular monitoring of *Salix* and *Vaccinium* by recordings of chlorophyll-a fluorescence then a yearly survey of vegetation cover by pin-point method were done in addition to an end season leaf harvest for determination of carbon, nitrogen, chlorophyll, and UV-B absorbing compounds. Much of the field work was performed before the start of the Ph.d. project, but much of the data analysis and writing was done in the early project period before the CLIMAITE manipulations took effort.

2.3 Photosynthetic performance characterization

The photosynthetic processes can be separated into energy producing (sources) and energy consuming processes (sinks) (See Box 2 in Paper IV). Source processes are involved in the capture of light and the processing through the photosynthetic apparatus resulting in available energy equivalents. Sink processes are the energy demanding processes, primarily CO₂-assimilation in the Calvin Cycle. The molecules of CO₂ diffuse into the leaf through stomatal openings into the stomatal cavities and from here further through the leaf mesophyll cells until finally reaching the chloroplast, where the Calvin Cycle takes place. Here, the CO₂ molecules assimilated are stored in energy rich metabolites i.e. sugar, which is allocated to different plant parts for growth and maintenance. Under field conditions, the often used methods to probe both the characteristics and activity of the source and sink sides are to do measurements of chlorophyll-a fluorescence and CO₂ and H₂O gas-exchange. The sink processes are evaluated by parameters as e.g. net photosynthesis (P_n), stomatal conductance (g_s) and transpiration (T_r), while the source processes are evaluated by a range of other parameters related

both to the handling of light in the photosynthetic apparatus and partitioning of energy fluxes (Schreiber 2004, Strasser *et al.* 2004). From fluorescence induction curves the e.g. maximal quantum yield (F_V/F_M), performance indexes (PI) (Strasser *et al.* 2004, see also definitions in Paper V) and from light curves with pulse amplitude fluorimetry the e.g. maximal quantum yield in light (F_V'/F_M'), Stern-Volmer non-photochemical quenching (NPQ), photochemical and non-photochemical quenching (qP and qN) (Schreiber 2004, see also definitions in Paper VII). We used programming in SAS to process the chlorophyll fluorescence measurements (more than 55000) and built in re-calculation and data validation steps. Use of simultaneous leaf gas exchange and chlorophyll-a fluorescence can be applied to relate the electron transport from light harvesting to the consumption of energy equivalents in the Calvin Cycle (Genty *et al.* 1989).

We used the most frequently used method for understanding how C3 photosynthesis at the leaf scale responded to the experimental manipulations, the FvCB model (Farquhar, von Caemmerer & Berry 1980). We adopted the approach by Dubois *et al.* (2007) for processing the data in SAS (more than 2500 A/C_i curves) and also built in leak correction (empty chamber approach as in Bernacchi *et al.* 2001, 2002, Manter and Kerrigan 2004), re-calculation and data validation steps. In the FvCB model, the biochemical reactions of photosynthesis are considered to be in one of two distinct steady states. In one state, the rate of photosynthesis can be predicted by the properties of ribulose 1.5-bisphosphate carboxylase/oxygenase (Rubisco) assuming a saturating supply of substrate, RuBP. This state is called Rubisco-limited photosynthesis and occurs when the concentration of CO₂ is low. In this state the photosynthesis is determined by the maximal velocity for Rubisco carboxylation, V_{cmax} . In the other state, photosynthetic rates are predicted assuming that the rate of regeneration of RuBP is limiting and so RuBP is used at a constant rate. This is called RuBP regeneration-limited photosynthesis and occurs at higher CO₂ concentration. In this state the photosynthesis is determined by the RuBP-regeneration, J_{max} . RuBP-regeneration-limited photosynthesis includes the conditions where light intensity limits the rate of photosynthesis but can also include conditions in which enzymes of the Calvin

cycle (other than Rubisco) limit the rate of photosynthesis. This third state is called triose-phosphate-use (TPU) limitation. In this condition, photosynthesis does not respond to increasing CO₂, nor is it inhibited by increasing oxygen concentration (Sharkey 1985) and is not considered in this thesis. Although widely used the approach of measuring A/C_i curves may lead to some variability in parameterizations as the method is not perfect (Long and Bernacchi 2003) due to e.g. leaks (Bernacchi *et al.* 2001, 2002, Flexas *et al.* 2007, Manter and Kerrigan 2004), edge effects (Jahnke and Krewitt 2002, Pons and Welschen 2002), influence of mesophyll conductance (Bernacchi *et al.* 2002, Flexas *et al.* 2002, 2004, Evans *et al.* 1986, Harley *et al.* 1992, Loreto *et al.* 1992), stomatal patchiness (Buckley 2005, Buckley *et al.* 2003, Terashima 1992), erroneous leaf temperature (Tyree and Wilmot 1990, Verhoef 1997) and stress full conditions with very low stomatal conductance (Boyer *et al.* 1997, Flexas *et al.* 2002, Galmes *et al.* 2006, Long and Bernacchi 2003). In the following the term photosynthetic capacity refers to V_{cmax}, J_{max} and the light and CO₂ saturated photosynthesis P_{max}. Under field conditions the light saturated photosynthesis is referred to as P_n (in ambient plots 380 ppm CO₂ and in FACE plots 510 ppm CO₂).

3. Photosynthetic performance and climate change

In the following the response of the studied heat plants, *Calluna vulgaris* and *Deschampsia flexuosa* are summarized based on studies in the following papers, referred to by their Roman numerals in the following.

3.1 Species characteristics and their growth strategy

Calluna vulgaris is a slow growing evergreen shrub classed as a stress tolerant competitor (Grime *et al.* 1988) having adaptive traits for dry and nutrient low habitats e.g. xeromorphic leaves, long leaf life span, low nitrogen content, low nutrient loss and likely low photosynthetic rates (Aerts 1995). The grass *Deschampsia flexuosa* has a opportunistic growth patterns with rapid leaf growth, high leaf nitrogen content (Grime *et al.* 1988) and likely high photosynthetic rates.

It has rhizomes (Scurfield 1954) wherefrom carbon and nutrient allocation to leaves take place in growth periods and serving as storage in dormant periods (Aerts 1993). *Deschampsia* has a maximal leaf life span less than a year, but there are always some green leaves, giving the species a 'semi-evergreen' character (Aerts 1993). It has a variable number of leaf cohorts per shoot which likely allow a large degree of flexibility to initiate or extend growth in response to environmental changes.

The differences between the two species growth pattern and plant eco-physiological responses to natural drought and autumn warming were studied in Paper I. The water availability exerted a large degree of control on the photosynthetic rates in un-manipulated *Calluna* and *Deschampsia* and the photosynthesis rates and leaf nitrogen content were highest in *Deschampsia*. Both species decreased photosynthesis during drought via decreased stomatal conductance and intercellular CO₂ concentration, but while *Calluna* shoots remained green the *Deschampsia* leaves died back. The total green leaf length per vegetative shoot flowered two growth phases, with maximum length just before flowering in early summer and a second after flowering in late summer (Paper I). No comparable leaf physiology studies have to our knowledge been made. Instead many conducted studies of *Deschampsia* are related to nutrient additions and competition with other species (e.g. Aerts *et al.* 1990, Aerts and Chapin 2000, Alonso and Hartley 1998, Britton *et al.* 2003). The present studies of *Calluna* supplements earlier short term acclimation studies where experimental drought decreased the plant water potential (Jackson *et al.* 1995) and decreased light saturated net photosynthesis, transpiration and stomatal conductance (Gordon *et al.* 1999a, 1999b). A gradient study across Europe showed that precipitation was a major factor controlling the magnitude of *Calluna* photosynthesis (Llorens *et al.* 2004). The two species increased the photosynthetic rates significantly immediately after rewetting, both via increased stomatal conductance and intercellular CO₂ concentration (Paper I). After the drought *Deschampsia* sprouted new leaves and *Calluna* grew new shoots with high nitrogen content and balanced C/N ratio. The similarity of photosynthetic response was in contrast to the different

phenological strategies of the dwarf shrub and the grass. This adds to the need for including both physiology and phenology when primary productivity patterns are to be understood and up-scaled in models (Paper I).

The unusual high temperature and sufficient water conditions allowed for high photosynthetic activity in both species in late autumn, extending the growing season (Paper I). A high leaf nitrogen concentration in both species indicate their potential for high autumn productivity in response to the favorable environmental conditions (Paper I)

Previously attempts to structure eco-physiological responses from plant functional types have been made (Arft *et al.* 1999, Chapin *et al.* 1996, Walker *et al.* 2006). Some studies find dwarf shrubs to be less responsive to environmental perturbations and the opportunistic grasses to be more responsive (Aerts 1995, Chapin and Shaver 1996, Graglia *et al.* 2001, Michelsen *et al.* 1999). Such differences could be due to differences in photosynthetic capacity characteristics (P_{\max} and J_{\max}) and carbon sink strength and also vary during growing season. We therefore used the factorial design to test these issues (Paper IV) and focused on whether responses to elevated CO_2 , warming and drought were additive or synergistic. The warming during spring and early season improved the photosynthetic capacity in both species and this underpin the potential for the productivity increases in response to autumn warming discussed above (Paper IV). This unusual warm autumn with frequent precipitations stimulated light saturated net photosynthesis, particular in elevated CO_2 , and postponed senescence beyond October (Paper III and IV), but warming as a single factor only caused a marginal effect on photosynthetic capacity and only in *Deschampsia* (Paper IV). Temperature was unusual high during September-November 2006 (DMI), and therefore the impact on photosynthetic capacity of the experimental passive nighttime warming was probably not as strong as it would have been if a large number of cold nights had occurred.

Growth strategy differences very much influenced the responses of the two species, e.g. when warming was combined with elevated CO_2 the opportunistic *Deschampsia* improved photosynthetic capacity even further concurrently with

luxurious water consumption via increased stomatal conductance (Paper IV). This response was reversed when soil water content became unfavorable. *Deschampsia* responded with accelerated leaf senescence and synergistically decreased photosynthetic capacity when warming was combined with elevated CO₂, and a major part of the leaves wilted (Paper IV). In contrast, the xeromorphic leaves of *Calluna* maintained metabolism with decreased photosynthetic capacity and light saturated photosynthesis in the drought period (Paper IV). The *Calluna* photosynthetic capacity was little responsive and only after severe drought stress the photosynthetic capacity was decreased in elevated CO₂ (Paper IV). Experimental drought also led to pronounced lowered plant water potential, stomatal conductance and light saturated net photosynthesis in *Calluna* (Paper III). This is clearly demonstrating the drought tolerance in this species although some decrease in green-to-brown ratio was seen (Paper III). Rewetting caused *Deschampsia* leaves to sprout new second phase leaves with high nitrogen uptake and photosynthetic capacity, especially in the previous drought treated plots (Paper IV) and these had high photosynthesis rates (Paper II). In contrast more rigid photosynthetic capacity regulation were seen in *Calluna*, taking advantage of increased photosynthesis per ground area as it maintained its canopy and kept the shoot metabolism active (Paper IV). Thus, the water relations clearly modulated the photosynthetic capacity responses to warming and elevated CO₂ and had a strong concurrent impact on N-availability (Paper IV). Although the species similarities in photosynthetic response contrasted to the different growth patterns, then the photosynthetic capacity characteristics in the experimental treatments seems to be adjusted in accordance with the different growth strategies of the dwarf shrub and the grass.

3.2 Warming influence on growing season length

Across a range of warming experiments with different approaches and in different ecosystems increases in measures of aboveground productivity have been found (Arft *et al.* 1999, Rustad *et al.* 2001). Along a European gradient the productivity stimulation in response to passive nighttime warming was

demonstrated to depend on water availability (Llorens *et al.* 2004, Peneuleas *et al.* 2007). Responses in these studies were argued to result from direct effects via increased photosynthesis at higher temperature, longer growing season or from indirect effects of increased nutrient and water availability (Arft *et al.* 1999, Llorens *et al.* 2004, Peneuleas *et al.* 2007, Rustad *et al.* 2001). In our study the warming treatment increased the potential for stimulated growth and advanced spring. For both *Calluna* and *Deschampsia* the spring photosynthesis was synergistically stimulated by the combination of warming and elevated CO₂ (Paper II and III). Further, both species had significant higher photosynthetic capacity (P_{\max} and J_{\max}) and as the V_{\max} was not affected by the warming treatment in early season this indicates that no carbon sink limitation of photosynthesis was apparent (Paper IV). Shaver *et al.* (2000) provides a conceptual framework for analyzing effects of warming on ecosystem processes and argues the influence of indirect effects to increase the complexity when cause-effect understanding are to be made. In our case it is not unlikely that part of the impact of warming in this period is mediated via shift in phenology, which warming have been reported to affect previously (Jentsch *et al.* 2008, Menzel *et al.* 2006, Penuleas *et al.* 2007). As stimulated productivity was to be expected in response to elevated CO₂ (Ainsworth and Long 2005, Ainsworth and Rogers 2007, Drake *et al.* 1997), it is noteworthy that warming and elevated CO₂ acted synergistically and effects thus could not be deduced from the single factor expectations.

The temperatures were unusual high during September-November 2006 (DMI), and the impact of the experimental passive nighttime warming was probably not as strong as it would have been if a large number of cold nights had occurred. Together with sufficient soil water content these conditions prolonged the growing season, leading to the highest growing season net photosynthesis figures in October (Paper I). Also in the autumn, the warming and elevated CO₂ interaction synergistically stimulated plant carbon uptake in *Calluna* (Paper III), but not in *Deschampsia* (Paper II). However, the late autumn increased photosynthetic capacity (J_{\max} and P_{\max}) point to some effect of warming in *Deschampsia* (Paper IV) and there were no effects on V_{\max} (Paper IV). These conditions and the

balanced leaf carbon uptake and nitrogen supply clearly increased the carbon sink strength of the vegetation and lengthening of spring and autumn have strong impacts on the yearly productivity, especially when warming is combined with enhanced CO₂ (Paper III). Sink strength stimulation can have several direct and indirect effects. Direct effects of the night time warming treatment during day is possible as the effect still evident 3–5h after sunrise (Mikkelsen *et al.* 2008). In principle this could provide closer to optimal growth temperature in warmed plots, and increase photosynthetic rates (Sage and Kubien 2007), but also respiration (Atkin and Tjoelker 2003). However, acclimation of respiration to temperature may occur (Atkin and Tjoelker 2003). More likely and in line with other studies the experimental warming caused indirect effects via altered growing season length and phenology (Harte and Shaw 1995, Wan *et al.* 2005) and increased soil nitrogen mineralization and availability (Peterjohn *et al.* 1994, Rustad *et al.* 2001). The increased sink strength may also be related to increased carbon cycling in part via increased nighttime leaf respiration (Turnbull *et al.* 2002, 2004), soil respiration (Piao *et al.* 2007, Schindlbacher *et al.* 2009) or other carbon consuming processes.

3.3 Productivity increases in elevated CO₂

Elevated CO₂ increased the net photosynthesis in *Calluna* and in *Deschampsia* via increased intercellular CO₂ concentration and the water use efficiency during most months (Paper II and III). Contrary to expected no decreased stomatal conductance was seen, even when water availability were progressively decreasing in *Calluna* and *Deschampsia* and actually even increased stomatal conductance in *Deschampsia* were seen when soil water availability were sufficient. This clearly demonstrates the improved water use efficiency to be determined by the increases in photosynthesis to a larger degree than decreases in water consumption. The increased carbon input in general increased the C/N ratios in response to elevated CO₂. Some explainable deviances from this pattern are e.g. the newly emerged *Deschampsia* leaves in August, not yet having accumulated enough carbon, but this reversed later on when photosynthesis was stimulated in elevated CO₂ (Paper II). The stimulation of nutrient uptake in the

former drought plots in autumn decreased C/N ratio and led to the synergistically decrease in C/N ratio by elevated CO₂ and drought (Paper II). For *Calluna* the C/N ratio almost doubled across treatments from May to July and was further increased in elevated CO₂, but when water availability increased in autumn increased nitrogen supply, likely via stimulated mineralization, balanced the C/N ratio in elevated CO₂ and also in all other treatments (Paper III). These patterns demonstrate that concurrent stimulation from water and nutrients do concur and therefore may be difficult to separate in the long term. The increased carbon sequestration in elevated CO₂ could be advantageous allocated to root growth as water shortage clearly decreases the magnitude of photosynthesis. Such a response could explain the synergistic stimulation of *Deschampsia* photosynthesis in response to warming and elevated CO₂ in August (Paper II). In opposition, a marginal synergistic decreased in *Calluna* photosynthesis was seen when warming and elevated CO₂ were combined in August (Paper III). Perhaps the opportunistic *Deschampsia* with marked higher WUE do respond faster than *Calluna* and consumes more soil water and more effectively. However, the occurring new *Deschampsia* leaves in August did sprout earlier in response to warming and elevated CO₂ as a synergistic decrease in $\delta^{13}\text{C}$ were seen (Paper II) pointing to potential allocation of carbon input to e.g. roots. Also the leaves may to a larger degree sprout where soil conditions are beneficial why our measurements may be affected by measuring on particular vital leaves.

In line with the robust response of increased photosynthesis and decreased stomatal conductance then the photosynthetic capacity measures as V_{cmax} and to a smaller degree also J_{max} and P_{max} are reported to decrease. With our data the carbon sink strength, as indicated by the V_{cmax} was negatively affected by drought in both species, but in response to elevated CO₂ as a single factor the decreases were only tendencies in *Calluna* whereas significant decreases were seen in *Deschampsia* (Paper IV). The photosynthetic capacity measures (J_{max} and P_{max}) were more flexible in *Deschampsia* showing decreases in elevated CO₂, and more rigid in *Calluna* (paper IV). As earlier argued this was in part due to strategy

differences, but also the pronounced nitrogen allocation to rewetted *Deschampsia* leaves increased the photosynthetic capacity (paper IV).

The response patterns of photosynthetic capacity and light saturated photosynthesis clearly demonstrate the increased carbon sequestration in elevated CO₂ to be limited by water availability in summer periods and the spring and autumn stimulation to be synergistically increased when combined with warming, while the impact of more frequent summer precipitations likely will increase productivity in elevated CO₂.

In the longer term nitrogen limitation very likely constrains the potential for increased productivity in response to CO₂ (Lou *et al.* 2004, Finzi *et al.* 2002, Hungate *et al.* 2006, Reich *et al.* 2006), but in this dry ecosystem it may difficult to separate this effect from the ample water conditions. When water apparently are such an important driver of the photosynthesis, how will the two different growth forms then respond to elevated CO₂ in the long term? We speculate that the long lived evergreen *Calluna* biomass and LAI likely tend to be adjusted to ensure sustainable water consumption and therefore the excess carbon to be allocated to other sinks thereby controlling the magnitude of photosynthesis in the long term. In contrast, the combination of the prevailing instantaneous nutrient, CO₂, light harvesting possibilities (LAI) and soil water conditions probable to a much higher degree limit the maximal standing green biomass in *Deschampsia* and therefore the long term responses may not be that different from the short term responses in this species.

The impact of events, of more extreme drought will have the most negative impact on *Deschampsia* in the short term, but in the long term will also affect *Calluna*. The stimulation of secondary compounds in *Calluna*, e.g tannins (Schmidth *et al.* 2007) may reduce the impact of herbivory (Freeman and Jantzen 1974, Iason and Hester 1993). Thus, herbivore attack followed by a severe drought in the *Calluna* community phase may be the basis for the most dramatic changes outcompeting the *Calluna*.

3.4 Importance of plant water relations

This paragraph focuses on how plant water consumption, drought and rewetting affect the photosynthetic performance. The natural drought decreased the soil water content and was accompanied by decreased stomatal conductance and reduced transpiration in both *Calluna* and *Deschampsia* (Paper I), in accordance with the expected association between soil water availability and stomatal conductance (Flexas and Medrano 2002, Schulze 1986). Under these conditions the stomatal conductance exerted a large degree of control of the magnitude of both species light saturated photosynthesis and where the *Calluna* shoots were maintained the *Deschampsia* leaves died back until rewetted in August (Paper I). These simple strategies caused complex response when treatments were applied.

For *Calluna*, elevated CO₂ stimulated photosynthesis, but did not affect stomatal conductance (Paper III). The experimental drought decreased the SWC in July and August, but not before August negative effects on net photosynthesis were measured, although the July water relations were clearly negatively affected (Paper III). Further, the *Calluna* plant water potential decreased in response to warming and drought in parallel with the SWC, but this did not affect stomatal conductance and transpiration (Paper III). However, warming and drought synergistically decreased the PWP, stomatal conductance and transpiration. Likely a higher leaf area index created in combination with the earlier start of the growing season in treatments combined with warming therefore increased water consumption and led to water shortage revealed by the T*D interaction. This may also explain the T*CO₂ interaction decreasing net photosynthesis in August (Paper III).

The *Deschampsia* responded to the progressively decreased soil water content with decreasing stomatal conductance (Paper I) and started to die back until rewetted in August. The photosynthetic capacity (P_{\max} and J_{\max}) improved in early growth season facilitated *Deschampsia* to synergistically increase the net photosynthesis when warming was combined with elevated CO₂ (Paper II and Paper IV). However, this was reversed when a large proportion of first phase

Deschampsia leaves wilted and synergistically decreased the P_{\max} and J_{\max} when warming were combined with elevated CO_2 . This also decreased the net photosynthesis and V_{cmax} (Paper II and Paper IV). The growth in the TCO₂ treatments was sustained via higher stomatal conductance and water consumption in June, but ceased in July where SWC decreased to the vicinity of the wilting point (Paper II). Negative effects of water shortage were clearly demonstrated for both species in the experimental drought treatment with the early effects in *Deschampsia*. Already in July decreased photosynthetic capacity (J_{\max} and P_{\max}) were seen in *Deschampsia* and this continued in August. For both species the decreased net photosynthesis in July and August further demonstrated the reduced sink strength in response to experimental drought (Paper II and III). This highlights the opportunistic resource exploration of atmospheric CO_2 and water, which *Deschampsia* is capable of by combining pronounced photosynthetic capacity regulations with a strategy of wilting down when the water availability gets to low. In contrast, *Calluna* operates with a lower stomatal conductance and higher water use efficiency in combination with a relatively lower photosynthetic capacity resulting in lower photosynthetic rates, but maintaining active photosynthetic metabolism throughout the drought period (Paper III).

The stimulation of photosynthesis and stomatal conductance decreases are very robust responses in elevated CO_2 (Ainsworth and Long 2005, Ainsworth and Rogers 2007, Medlyn *et al.* 2001) and this may result in reduced soil water depletion that can mitigate negative effects of drought and sustain net photosynthesis in dry periods (Hungate *et al.* 1997, Leuzinger and Körner 2007, Morison and Gifford 1984, Robredo *et al.* 2007). Surprisingly, our data did not demonstrate stomatal conductance decreases in elevated CO_2 and the $\delta^{13}\text{C}$ cannot reveal if such decreases potentially occurred between campaigns as the industrial CO_2 dosed in FACE plots differ from the atmospheric signature. The reason for stomatal conductance reduction is not demonstrated is maybe related to the chosen methodology, not being very well suited to register e.g. midday depression in stomatal conductance.

Concerning 'water savings' the SWC in 0-60 cm was higher in elevated CO₂ plots (Paper III) compared to ambient plots during summer and although this was not found in 0-20 cm (Paper II) this clearly suggest an improved water economy for plants in elevated CO₂. Likely the water consumption in the 0-20 cm zone, where the majority of both species roots are distributed, depleted this compartment. Also the earlier start of the growing season in response to warming leads to increased water consumption causing negative impact on water availability later on, most pronounced in the experimental drought plots. However, some mechanism should explain the observed soil water savings. Long term changes in the ratio of leaf to sapwood area may occur in *Calluna* (Jackson *et al.* 1999) as in other woody plants (Margoils *et al.* 1995, Mencuccini and Grace 1995), but the increased SLA and carbon uptake in elevated CO₂ and the decreases in experimental drought as well as the short duration of the manipulations clearly suggest that this did not take place. The *Deschampsia* wilted a major proportion of leaves in response to low water availability (Paper I and II), this may increase evaporation in ambient conditions to a larger degree than under elevated CO₂, leading to the higher SWC in elevated CO₂. The LAI changes will affect the evapo-transpiration, so when reduced, an exacerbated soil moisture loss via increased heat load and evaporation at the soil surface will take place (Obrist *et al.* 2003, Wan *et al.* 2002). As discussed in the introduction the opposite may happen as loss of soil moisture in warm and dry conditions may be slowed if declines in LAI also occur (Wan *et al.* 2002, Zavaleta *et al.* 2003). This add to the need for a better understanding of the coupling between plant growth pattern and the resulting LAI influence on soil moisture contents, and improved understanding is crucial to model future ecosystem productivity (Cowling and Field 2003, Ewert 2004, Filella *et al.* 2004). It is noteworthy that even if stomatal conductance decreased this does not necessarily translate into reduced transpiration as leaf temperature, wind speed, vegetation roughness and density, vapor pressure deficit, atmospheric temperature, humidity and pressure are factors that may increase the water loss from plant surface and also soil (Amthor 1999, Morgan *et al.* 2004, Field *et al.* 1995, Jacobs and de Bruin 1997).

3.5 Ambient ultra-violet radiation impacts in high arctic heath plants

We hypothesized that if the present UV radiation affects the vegetation significantly, then reduction of the irradiance load would improve the photosynthetic performance of the plants. Therefore an experiment where ambient UV-irradiance is reduced substantially by means of filters was established in NE-Greenland. This approach allows directly results to the impact of the current level of UV-radiation, including the variability of exposure during the growing season and from year to year. Earlier studies in the area, with similar setup, have reported reduced root biomass in *Vaccinium uliginosum*, and changes in microbial community composition in ambient UV-B (Rinnan *et al.* 2005). We have also found decreased PSII performance in spite of increased UV-B screening compounds in *Salix arctica* and *Vaccinium uliginosum* (Albert *et al.* 2005, Bredahl *et al.* 2004). Later studies focused on linking PSII performance to net photosynthesis (Paper V, Paper VII), long term responses (VIII) and summarizing initial findings (Paper VII).

From the range of UV-exclusion experiments conducted at Zackenberg we observe several significant plant-ecophysiological responses (Paper VI, Table 1). The chosen parameters, i.e. photosynthesis and probing of plant stress, are generally expected to respond faster than many other important ecological processes such as growth, phenology and species composition, which may respond to disturbances on a longer timescale (Callaghan *et al.* 2004c). However, inter-species differences in plant performance characteristics are important since the traits are likely to influence competition and the resulting plant cover.

The measurements of chlorophyll-a fluorescence induction curves led to calculation of the much reported parameter, maximal quantum yield (F_v/F_m), which closely relates to Photosystem II (PSII) function and is often interpreted as a proxy for plant stress related to photosynthetic performance (Strasser *et al.* 2004, see Box 2 in Paper VII). Also the so-called Performance Index (PI, Strasser *et al.* 2004) was derived from the fluorescence measurements. The PI integrates into one parameter the proportional responses of energy fluxes related to trapping and dissipation within the PSII and also to the energy transport behind PSII. Hereby the

PI expresses the overall effective energy processing through PSII and is believed to sum up the accumulative stress effects on PSII.

The UV-exclusion experiments were initiated in 2001 (see Box 1), and it was found that a reduction of the ambient UV-B level resulted in decreased content of UV-B absorbing compounds and lower stress level indicated by increased maximal quantum yield (F_V/F_M) and Performance Indexes (PI) (Paper VII and VIII) in arctic willow *Salix arctica* and Bog blueberry *Vaccinium uliginosum*. Moreover, the analysis of leaf level gas exchange revealed a decreased stomatal conductance and internal CO_2 -concentration in *Salix arctica* when ambient UV-B was reduced (Bredahl *et al.* 2004). These findings were initially concluded to indicate the important impact of UV-B in the short term. However, the greatly varying leaf angle results in differences in UV-B doses for the individual leaves, which probably confounded the effects. This hypothesis led to an experimental attempt to homogenize the UV-B dose received by the leaves by manipulative fixation of the plant leaves perpendicular to the sun. The second season (2002) included such a setup with fixation of leaves, and this led to clear-cut positive impacts on almost all measured and derived fluorescence parameters on *Salix arctica*. The results on F_V/F_M and PI confirmed that ambient UV-B radiation is a significant plant stressor (Albert *et al.* 2005). Also the proportions of energy fluxes per leaf cross section were quantified, and the dissipation of untrapped energy was highest under ambient UV-B resulting in significantly lower flux of energy beyond the electron intersystem carriers (Albert *et al.* 2005). These responses are argued to be specifically due to the UV-B radiation (Albert *et al.* 2005), and they demonstrate a less effective energy processing in the photosynthetic machinery. For logistical reasons no leaf-level photosynthetic measurements were done on *Salix arctica* that year, but in order to relate results directly to other more traditional photosynthetic parameters, e.g. quantum yield, non-photochemical quenching, maximum rate of electron transport, maximum rate of Rubisco carboxylation and photosynthetic light response, we combined gas-exchange and fluorescence measurements simultaneously on *Salix arctica* in 2005 (Paper VII). In addition we investigated the importance of manipulating the leaf exposure, the PSII

performance was followed in parallel through most of the growing season on two additional sites with leaves in their natural leaf angle positions in the vegetation cover. This study demonstrated that ambient UV-B reduced net photosynthesis in high arctic *Salix arctica* when ambient irradiance doses were maximized. The simultaneous gas-exchange and fluorescence measurements revealed that ambient UV-B decreased both Calvin Cycle capacity via maximum rate of electron transport and maximum carboxylation rate of Rubisco and the PSII performance via decreased quantum yield and increased energy dissipation processes. The parallel decreased PSII performance for *Salix arctica* on three sites (on level ground, sloping and south facing, fixed angle site) a level of varying exposure signifies this response to be extended to a large proportion of leaves across random position and angle in the vegetation (Paper VII). The study on *Vaccinium uliginosum* extends the response pattern to this species also (Paper V). The *Vaccinium uliginosum* plants showed a decreased photosynthesis in parallel with decreased values of F_v/F_m and PI in ambient UV-B compared to the reduced UV-B treatment (Paper V). This response was seen through most of the growth season, but in the senescence period in late August the treatment differences disappeared. These results clearly linked the decrease in net photosynthesis to the stress effects on the light energy harvesting and processing machinery. Further, the clear detection of negative impacts on PSII performance across the great variation of natural leaf angles and years clearly points to ambient UV-radiation as an important stressor in high arctic plants. Even higher impact is deduced by increasing natural UV-radiation doses, as demonstrated in practice by maximizing the ambient UV-B level by Albert *et al.* (2005) and in Paper VII. This may not only be mediated by changes in ozone layer thickness and cloud cover alone, since an earlier onset of spring will expose vegetation to the higher UV-B spring fluxes in June. The long term response revealed that even though plants had a higher level of UV-B absorbing compounds in the leaves under ambient UV-B the possible protective screening by these compounds were not sufficient to avoid negative effects on the photosynthetic machinery (Paper VIII). These findings add further

evidence that ambient UV-B is an important stress factor for plants in high arctic heath.

The range of significant responses seen in the UV-B exclusion experiments at Zackenberg clearly indicates that ambient UV-B is a plant stress factor in this area (Paper VI). This seems in contrast to the reported robustness towards supplemental UV-B for plants in sub-arctic Abisko and high-arctic Svalbard. However, the results from UV-B exclusion studies in the Antarctic region have demonstrated effects on plants, similar to the results from Zackenberg. There are differences in the chosen response variables, and the contrasting responses may be interpreted to be due to the climatic differences between the areas. Further, the extreme living conditions in the high-arctic Zackenberg and Antarctic region may to a larger degree amplify effects of the stress factors, leading to significant UV-B impacts here. Although the responses from Zackenberg provides new insight and supplements earlier work, more work dedicated to link variables across scales are needed to take full advantage of the earlier findings. Thus, only by making parallel UV-B supplementation and UV-B exclusion field experiments it is possible to exclude the methodological differences and validate the ecosystem responses. Furthermore, the experiments should be conducted over longer time periods and include more traditional parameters (e.g. shoot growth rate and biomass effects) in order to ease comparisons and to elucidate whether the observed changes are transient or whether they accumulate over years. Long-term experiments are especially important for valid determination of belowground responses, that have the potential to pose great feedbacks on the ecosystem functioning (Paper VI).

If projections from climatic scenarios to future biological responses shall be made, the biological responses and their feedback must be detected in multi-factorial experiments closely resembling the climatic projections. If sufficient reliable biological response functions to climatic parameters can be established then ecosystem modeling shall be possible. Presently, we have not sufficient knowledge of all responses of importance and their interactions. Concerning ozone layer depletion, a specific UV-B radiation scenario for Zackenberg is needed. What we can state is that ambient UV-B as a single factor affects plant life negatively at

high-arctic Zackenberg, and that the methodology developed is very well suited for long term monitoring.

4. Conclusions

- Elevated CO₂ increased net photosynthesis, but did not decrease stomatal conductance.
- Experimental drought decreased stomatal conductance and net photosynthesis
- Water availability exerted a large degree of control of photosynthesis and photosynthetic capacity, but in response to water shortage the evergreen shrub maintained its shoots where the opportunistic grass leaves wilted.
- Warming alone had only small effect on photosynthesis but when combined with elevated CO₂ this combination synergistically increased net photosynthesis in spring and autumn.
- Warming increased photosynthetic capacity in early spring in both species and in late autumn in the grass.
- Elevated CO₂ mitigated the negative impact of drought on photosynthesis rate per leaf area.
- Elevated CO₂ had only little effect on photosynthetic capacity in the dwarf shrub, but in the grass photosynthetic capacity levels were variable.
- Experimental drought decreased photosynthetic capacity in *Calluna* after severe water shortage, but elevated CO₂ synergistically mitigated this.
- Experimental drought decreased photosynthetic capacity in the remaining *Deschampsia* leaves, but when rewetted pronounced photosynthetic capacity increase was seen.
- Growth strategy very much influenced the eco-physiological responses to environmental perturbations.
- Summer photosynthesis stimulation is most likely if precipitations allow for sufficient water conditions.
- Autumn photosynthesis stimulation is most likely in warm years when water conditions are sufficient too.

- The treatment that best represent the future climate increased the productivity in early and late season.
- The concerted impact of changes in temperature, precipitation and atmospheric CO₂ affects net photosynthesis and have different strength and impact during season and also interact in a way that could not be deduced from single factors experimentation.
- Warming increased the photosynthetic capacity in spring.
- Despite increased screening of UV-B absorbing compounds the photosynthetic performance was decreased in high arctic heath plants.
- PSII performance was decreased in ambient UV-B in high arctic heath plants both in short and in the long term.
- Net photosynthesis was decreased concurrently with PSII performance in ambient UV-B.
- Current UV-B levels have negative effect on photosynthetic performance in high arctic heath plants, which may be further enhanced in years with advanced spring in response to warming.

5. Perspectives

- Difference in response time in the ecosystem pools and processes may feedback to photosynthesis and therefore the conditions for our one year responses may change. Therefore long term studies are needed to validate and consolidate our findings at both the CLIMAITE site and on the Arctic sites.
- As the water relations exert a great degree of control of growth responses and photosynthesis magnitude, the influence of precipitation patterns and amounts in the long term will be of importance for the carbon and water fluxes.
- The variable leaf growth and photosynthetic performance in combination with the opportunistic resource consumption of the grass may influence the yearly per ground area carbon and water fluxes in a very variable way, whereas the dwarf shrub influence may vary less.

- Modeling of primary productivity based on up-scaling the leaf gas exchange measurements are to be performed, but the dependence of leaf area index and phenology and vice versa likely affects the prediction sensitivity and this further add to the need for high resolution data across the studied scales.
- Future investigations should aim at conducting high resolution and preferentially continuous measurements of leaf gas-exchange on the same units throughout season to separate physiological and phenological effects, validate model predictions of CO₂ and H₂O fluxes, and reveal the treatment influence of variations during both day and night. Also a better understanding of the temporal variation of H₂O fluxes between soil compartments, species consumption and evaporation is needed.
- Treatments and their impacts depend on the variation in climate thus their strength may affect the year to year responses to a different degree. The impact of the interactions may therefore also vary.
- The chosen parameters for studying ambient UV-B impacts revealed robust responses and has been approved for long term monitoring at field sites in High arctic Zackenberg and Subarctic Nuuk.
- The maintenance of the platforms for ambient UV-B studies potentially will reveal long term responses and comparisons of sub-arctic versus high arctic changes, but should ideally be combined with other environmental changes of importance e.g. season length, warming, elevated CO₂, precipitation etc.

References

- Acock B, Allen LH. 1985.** Crop responses to elevated carbon dioxide concentrations. *In*: Strain BR, Cure JD (eds) Direct effects of increasing carbon dioxide on vegetation. Office of Energy Research, Department of Energy, Washington, pp 53–97.
- Aerts R. 1989.** Above ground nutrient dynamics of *Calluna vulgaris* and *Molinia caerulea* in a dry heathland. *Oikos* **56**: 31-38.
- Aerts R, Berendse F, de Caluwe H, Schmitz M. 1990.** Competition in heathland along an experimental gradient of nutrient availability. *Oikos* **57**: 310-318.
- Aerts R. 1993.** Biomass and nutrient dynamics of dominant plant species in heathlands. *In*: Heathlands: Patterns and Processes in a changing environment. Aerts R, Heil GW. (Eds.), Geobotany, 20, Kluwer Academic Publishers, pp51-84.
- Aerts R. 1995.** The advantages of being evergreen. *Tree* **10**: 402-406.
- Aerts R, Chapin III SF. 2000.** The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research* **30**: 1-67.
- Arft AM, Walker MD, Gurevitch J, Alatalo JM, Bret-Harte MS, Dale M, Diemer M, Gugerli F, Henry GHR, Jones MH, Hollister RD, Jónsdóttir IS, Laine K, Lévesque E, Marion GM, Molau U, Mølgaard P, Nordenhäll U, Raszhivin V, Robinson CH, Starr G, Stenström A, Stenström M, Totland Ø, Turner PL, Walker LJ, Webber PJ, Welker JM, Wookey PA. 1999.** Responses of tundra plants to experimental warming: Meta-analysis of the International Tundra Experiment. *Ecological Monographs* **69**: 491-511.
- Ainsworth EA, Davey PA, Hymus GJ, Osborne CE, Rogers A, Blum H, Nosberger J, Long SP. 2003.** Is stimulation of leaf photosynthesis by elevated carbon dioxide concentration maintained in the long term? A test with *Lolium perenne* grown for 10 years at two nitrogen fertilization levels under Free Air CO₂ Enrichment (FACE). *Plant Cell and Environment* **26**: 705-714.
- Ainsworth EA, Long S. 2005.** What have we learned from 15 years of Free-Air CO₂ Enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New Phytologist* **165**: 351-372.
- Ainsworth EA, Rogers A. 2007.** The response of photosynthesis and stomatal conductance to rising [CO₂]: mechanisms and environmental interactions. *Plant, Cell and Environment* **30**: 258-270.

Albert KR, Mikkelsen TN, Ro-Poulsen H. 2005. Effects of ambient versus reduced UV-B radiation on high arctic *Salix arctica* assessed by measurements and calculations of chlorophyll-a fluorescence parameters from fluorescence transients. *Physiologia Plantarum* **124**: 208-226.

Albert KR, Mikkelsen TN, Ro-Poulsen H. 2008a. Ambient UV-B radiation decreases photosynthesis in high arctic *Vaccinium uliginosum*. *Physiologia Plantarum* **133**: 199-210.

Albert KR, Rinnan R, Ro-Poulsen H, Mikkelsen TN, Håkansson KB, Arndal MF, Michelsen A. 2008b. Solar Ultraviolet-B Radiation (UV-B) at Zackenberg: The Impact on Higher Plants and Soil Microbial Communities. In: Møller H, Christensen T, Elberling B, Forchhammer M, Rasch M, (eds.), Dynamics of a High Arctic Ecosystem, New York, USA, Elsevier. *Advances in Ecological Research* **40**: 421-440.

Alonso I, Hartley S. 1998. Effect of nutrient supply, light availability and herbivory on the growth of heather and three competing grass species. *Plant Ecology* **137**: 203–212.

Allen DJ, McKee IF, Farage PK, Baker NR. 1997. Analysis of limitations to CO₂ assimilation on exposure of leaves of two *Brassica napus* cultivars to UV-B. *Plant, Cell and Environment* **20**: 633–640.

Allen DJ, Nougès S, Morison JIL, Greenslade PD, Mcleod AR, Baker NR. 1999. A thirty percent increase in UV-B has no impact on photosynthesis in well-watered and droughted pea plants in the field. *Global Change Biology* **5**: 235-244.

Amthor JS. 1999. Increasing atmospheric CO₂, water use, and water stress: scaling up from the plant to the landscape. In: Carbon Dioxide and Environmental Stress (eds. Lou Y, Mooney HA), Academic press, San Diego, pp 33-59.

Andresen L.C, Michelsen A. 2005. Off-season uptake of nitrogen in temperate heath vegetation. *Oecologia* **144**: 585-597.

Arft AM, Walker MD, Gurevitch J, Alatalo JM, Bret-Harte MS, Dale M, Diemer M, Gugerli F, Henry GHR, Jones MH, Hollister RD, Jónsdóttir IS, Laine K, Lévesque E, Marion GM, Molau U, Mølgaard P, Nordenhäll U, Raszhivin V, Robinson CH, Starr G, Stenström A, Stenström M, Totland Ø, Turner PL, Walker LJ, Webber PJ, Welker JM, Wookey PA. 1999. Responses of tundra plants to experimental warming: Meta-analysis of the International Tundra Experiment. *Ecological Monographs* **69**: 491-511.

Aro EM, Virgin I, Andersson B. 1993. Photoinhibition of Photosystem II in activation, protein damage and turnover. *Biochimica et Biophysica Acta* **1143**: 113–134.

Asada K. 2000. The water-water cycle as alternative photon and electron sinks. *Philosophical Transactions of the Royal Society of London B Biological Sciences* **29**: 1419–1431.

Atkin OK, Tjoelker MG. 2003. Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends in Plant Science* **8**: 343-351.

BassiriRad H. 2000. Kinetics of nutrient uptake by roots: responses to global change. *New Phytologist* **147**: 155-169.

Baker N, Nogués S, Allen DJ. 1997. Photosynthesis and photoinhibition. *In*: Lumsden PJ (ed.), *Plant and UVB: Responses to Environmental Change*. Society for Experimental Biology Seminar Series 64, Cambridge, UK: Cambridge University Press, 233–246.

Balleré CL, Scopel AL, Stapelton AE, Yanovsky MJ. 1996. Solar UV-B affects the seedling emergence, DNA integrity, plant morphology, growth rate, and attractiveness to herbivore insects in *Datura ferox*. *Plant Physiology* **112**: 161-170.

Balleré CL, Rousseaux MC, Searles PS, Zaller JG, Giordano CV, Robson TM, Caldwell MM, Sala OE, Scopel AL. 2001. Impacts of solar ultraviolet radiation on terrestrial ecosystems of Tierra del Fuego (southern Argentina) – an overview of recent progress. *Journal of Photochemistry and Photobiology B. Biology* **62**: 67-77.

Beier C, Emmett B, Gundersen P, Tietema A, Penuelas J, Estiarte M, Gordon C, Gorissen A, Llorens L, Roda F, Williams D. 2004. Novel approaches to study climate change effects on terrestrial ecosystems in the field: Drought and passive nighttime warming. *Ecosystems* **7**: 583-597.

Bernacchi CJ, Singaas EL, Pimentel C, Portis AR, Long SP. 2001. Improved temperature response functions for models of Rubisco-limited photosynthesis. *Plant, Cell & Environment* **24**: 253–259.

Bernacchi CJ, Portis AR, Nakano H, von Caemmerer S, Long SP. 2002. Temperature response of mesophyll conductance. Implications for the determination of Rubisco enzyme kinetics and for limitations to photosynthesis in vivo. *Plant Physiology* **130**: 1992–1998.

Bernacchi CJ, Morgan PB, Ort DR, Long SP. 2005. The growth of soybean under free air [CO₂] enrichment (FACE) stimulates photosynthesis while decreasing in vivo rubisco capacity. *Planta* **220**: 145-154.

Bernacchi CJ, Leakey ADB, Hady LE, Morgan PB, Dohleman FG, McGrath JM, Gillespie KM, Wittig VE, Rogers A, Long SP, Ort DR. 2006. Hourly and seasonal variation in photosynthesis and stomatal conductance of soybean grown

at future CO₂ and ozone concentrations for three years under fully open air field conditions. *Plant, Cell and Environment* **29**: 2077-2090.

Björn LO, Callaghan TV, Gehrke C, Gwynn-Jones D, Lee JA, Johanson U, Sonesson M, Buck ND. 1999. Effects of ozone depletion and increased ultraviolet-B radiation on northern vegetation. *Polar Research* **18**: 331-337.

Boyer JS. 1976. Photosynthesis at low water potentials. *Philosophical Transactions of the Royal Society B* **273**: 510-512.

Boyer JS. 1982. Plant productivity and environment. *Science* **218**: 443-448.

Boyer JS, Wong SC, Farquhar GD. 1997. CO₂ and water vapour exchange across leaf cuticle (epidermis) at various water potentials. *Plant Physiology* **114**: 185-191.

Bredahl L, Ro-Poulsen H, Mikkelsen TN. 2004. Reduction of the ambient UV-B radiation in the high arctic increases F_v/F_m in *Salix arctica* and *Vaccinium uliginosum* and reduces stomatal conductance and internal CO₂ concentration in *Salix arctica*. *Arctic, Antarctic, and Alpine Research* **36**: 364-369.

Bremer DJ, Ham JM, Owensby CE. 1996. Effect of elevated atmospheric carbon dioxide and open top chambers on transpiration in a tall grass prairie. *Journal of Environmental Quality* **20**: 691-701.

Britton A, Marrs R, Pakeman R, Carey P. 2003. The influence of soil-type, drought and nitrogen addition on interactions between *Calluna vulgaris* and *Deschampsia flexuosa*: implications for heathland regeneration. *Plant Ecology* **166**: 93-105.

Buckley TN. 2005. The Control of Stomata by Water Balance. *New Phytologist* **168**: 275-291.

Buckley TN, Mott KA, Farquhar GD. 2003. A hydromechanical and biochemical model of stomatal conductance. *Plant, Cell & Environment* **26**: 1767-1785.

Callaghan TV, Press MC, Lee JA, Robinson D, Anderson C. 1999. Spatial and temporal variability in the response of Arctic terrestrial ecosystems to environmental change. *Polar Research* **18**: 1-7.

Callaghan TV, Björn LO, Chernov Y, Chapin T, Christiansen TR, Huntley B, Ims RA, Johansson M, Jolly D, Jonasson S, Matveyeva N, Panikov N, Oechel W, Shaver G. 2004a. Uncertainties and recommendations. *Ambio* **33**: 474-479.

Callaghan TV, Björn LO, Chernov Y, Chapin T, Christiansen TR, Huntley B, Ims RA, Johansson M, Jolly D, Jonasson S, Matveyeva N, Panikov N, Oechel

W, Shaver G. 2004b. Rationale, concepts and approach to the assesment. *Ambio* **33**: 393-377.

Callaghan TV, Björn LO, Chapin T, Chernov Y, Christensen TR, Huntley B, Ims RA, Johansson M, Riedlinger Jolly D, Jonasson S, Matveyeva N, Oechel W, Panikov N, Shaver G. 2005. Arctic tundra and polar dessert ecosystems. *In*: Symon C, Arris L, Heal B, (Eds.) ACIA, Arctic Climate Impact Assessment. New York, USA: Cambridge University Press, pp243–352.

Caldwell MM. 1968. Solar ultraviolet radiation as an ecological factor in plant life. *Ecological Monographs* **39**: 243-268.

Caldwell MM, Robberecht R, Billings WD. 1980. A steep latitudinal gradient of solar ultraviolet-B radiation in the arctic-alpine life zone. *Ecology* **61**: 600-611.

Caldwell MM, Flint SD. 1994. Stratospheric ozone reduction, solar UV-B radiation and terrestrial ecosystems. *Climatic Change* **28**: 375-394.

Caldwell MM, Flint SD. 1997. Uses of biological spectral weighting functions and the need of scaling for the ozone reduction problem. *Plant Ecology* **128**: 66-76.

Castrillo M, Calcagno AM. 1989. Effects of water stress and rewatering on ribulose 1,5-bisphosphate carboxylase activity, chlorophyll and protein contents in two cultivars of tomato. *Journal of Horticultural Science* **64**: 717–724.

Chaves MM. 1991. Effects of water deficits on carbon assimilation. *Journal of Experimental Botany* **42**: 1–16.

Chaves MM, Maroco JP, Pereira JS. 2003. Understanding plant responses to drought – from genes to the whole plant. *Functional Plant Biology* **30**: 239–264.

Chapin FS III, Walker BH, Hobbs RJ, Hooper DU, Lawton JH, Sala OE, Tilman D. 1997. Biotic control over functioning of ecosystems. *Science* **277**: 500-504.

Chapin FS III, Shaver GR. 1996. Physiological and growth responses of Arctic plants to a field experiment simulating climatic change. *Ecology* **77**: 822-840.

Chapin III FS, Sydonia M B-H, Hobbie SE, Zhong H. 1996. Plant functional types as predictors of transient responses of arctic vegetation to global change. *Journal of vegetation science* **7**: 347-358.

Christensen JH, Christensen OB. 2003. Climate modeling: Severe summertime flooding in Europe. *Nature* **421**: 805-806.

Ciais P, Reichstein M, Viovy N, Granier A, Ogée J, Allard V, Aubinet M, Buchmann N, Bernhofer Chr, Carrara A, Chevallier F, De Noblet N, Friend AD,

Friedlingstein P, Grünwald T, Heinesch B, Keronen P, Knohl A, Krinner G, Loustau D, Manca G, Matteucci G, Miglietta F, Ourcival JM, Papale D, Pilegaard K, Rambal S, Seufert G, Soussana JF, Sanz MJ, Schulze ED, Vesala T, Valentini R. 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature* **437**: 529-533.

Classens AT, Langeley JA. 2005. Data-model integration is not magic: Modelling ecosystem responses to global change: Techniques and recent advances. *New Phytologist* **166**: 367-370.

Cleland EE, Chiariello NR, Loarie SR, Mooney HA, Field CB. 2006. Diverse responses of phenology to global changes in a grassland ecosystem. *Philosophical Transactions of the National academy of Science* **103**: 13740-13744.

Cowling SA, Field CB. 2003. Environmental control of leaf area production: implications for vegetation and landsurface modeling. *Global Biogeochemical Cycles* **17**: 1-14.

Cornic G, Massacci A. 1996. Leaf photosynthesis under drought stress. In: Baker NR, (ed.), *Photosynthesis and the Environment*. Dordrecht, the Netherlands: Kluwer Academic, pp347-366.

Curtis PS. 1996. A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. *Plant, Cell and Environment* **19**: 127-137.

Curtis PS, Wang X. 1998. A meta analysis of elevated CO₂ effects on woody plant mass, form and physiology. *Oecologia* **113**: 299-313.

Cybulski WJ, Peterjohn WR. 1999. Effects of ambient UV-B radiation on the above ground biomass of seven temperate-zone plant species. *Plant Ecology* **145**:175-181.

Day TA, Ruhland CT, Grobe CW, Xiong F. 1999. Growth and reproduction in Antarctic vascular plants in response to warming and UV-radiation reductions in the field. *Oecologia* **119**: 24-35.

Day TA, Ruhland CT, Xiong FS. 2001. Influence of solar ultraviolet-B radiation on Antarctic terrestrial plants: results from a 4-year field study. *Journal of Photochemistry and Photobiology B. Biology* **62**: 78-87.

Day TA, Neale PJ. 2002. Effects of UV-B radiation on terrestrial and aquatic primary producers. *Annual Review of Ecology and Systematics* **33**: 371-396.

Dermody O. 2006. Mucking through multifactor experiments; design and analysis of multifactor studies in global change research. *New Phytologist* **172**: 589-591.

Downton WJS, Loveys BR, Grant WJR. 1988. Non-uniform stomatal closure induced by water stress causes putative non-stomatal inhibition of photosynthesis. *New Phytologist* **110**: 503–509.

DMI.dk. Danish Meteorological Institute homepage:
http://www.dmi.dk/dmi/index/danmark/meteorologiske_ekstremer_i_danmark.htm

Drake B, Gonzalez-Meler M, Long S. 1997. More efficient plants: a consequence of rising atmospheric CO₂? *Annual Review of Plant Physiology and Plant Molecular Biology* **48**: 609-639.

Dubois J-JB, Fiscus EL, Booker FL, Flowers MD, Reid CD. 2007. Optimizing the statistical estimation of the parameters of the Farquhar-von Caemmerer-Berry model of photosynthesis. *New Phytologist* **176**: 402-414.

Dukes JS, Chiariello NR, Cleland EE, Moore LA, Shaw MR, Thayer S, Tobeck T, Mooney HA, Field CB. 2005. Responses of grassland production to single and multiple global environmental changes. *PLoS Biology* **3**: 1829-1837.

Ellsworth DS, Reich PB, Naumburg ES, Koch GW, Kubiske ME, Smith SD. 2004. Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO₂ across four free-air CO₂ enrichment experiments in forest, grassland, and desert. *Global Change Biology* **10**: 2121-2138.

Ehleringer J, Mooney HA. 1983. Productivity of desert and Mediterranean-climate plants. *In*: Lange OL, Nobel PS, Osmond CB, Ziegler H, (eds.), *Encyclopedia of Plant Physiology. Physiological Plant Ecology*, Vol. 12D. Berlin: Springer-Verlag, pp205–231.

Emmett BA, Beier C, Estiarte M, Tietema A, Kristensen HL, Williams D, Peñuelas J, Schmidt IK, Sowerby A. 2004. The response of soil processes to climate change: Results from manipulation studies of shrublands across an environmental gradient. *Ecosystems* **7**: 625-637.

Ennahli S, Earl HJ. 2005. Physiological limitations to photosynthetic carbonassimilation in cotton under water stress. *Crop Science* **45**: 2374–2382.

Evans JR, Sharkey TD, Berry JA, Farquhar GD. 1986. Carbon isotope discrimination measured concurrently with gas exchange to investigate CO₂ diffusion in leaves of higher plants. *Australian Journal of Plant Physiology* **13**: 281–292.

Ewert F. 2004. Modelling plant responses to elevated CO₂: How important is leaf area index? *Annals of Botany* **93**: 619–627.

Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO₂ carbon dioxide assimilation in leaves of C₃ carbon pathway species. *Planta* **149**: 78-90.

Filella I, Penuelas J, Llorens L, Estiarte M. 2004. Reflectance assessment of seasonal and annual changes in biomass and CO₂ uptake of a Mediterranean shrubland submitted to experimental warming and drought. *Remote Sensing of Environment* **90**: 308–318.

Field CB, Jackson RB, Mooney HA. 1995. Stomatal responses to increased CO₂: implications from the plant to the global scale. *Plant, Cell and Environment* **18**: 1214 - 1225.

Field CB, Lund CP, Chiariello NR, Mortimer BE. 1997. CO₂ effects on the water budget of grassland microcosm communities. *Global Change Biology* **3**: 197–206.

Finzi AC, Allen AS, Ellsworth DS, DeLucia EH, Schlesinger WH. 2001. Litter production, chemistry and decomposition following two years of free-air CO₂ enrichment. *Ecology* **82**: 470–484.

Finzi AC, DeLucia EH, Hamilton JG, Richter DD, Schlesinger WH. 2002. The nitrogen budget of a pine forest under free air CO₂ enrichment. *Oecologia* **132**: 567–578.

Flexas J, Medrano H. 2002. Drought-inhibition of Photosynthesis in C₃ plants: Stomatal and Non-stomatal limitations revisited. *Annals of Botany* **89**: 183-189.

Flexas J, Bota J, Escalona JM, Sampol B, Medrano H. 2002. Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. *Functional Plant Biology* **29**: 461–471.

Flexas J, Bota J, Loreto F, Cornic G, Sharkey TD. 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C₃ plants. *Plant Biology* **6**: 269–279.

Flexas J, Bota J, Galmés J, Medrano H, Ribas-Carbó M. 2006. Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. *Physiologia Plantarum* **127**: 343–352.

Flexas J, Diaz-Espejo A., Galmés J, Kaldenhoff R, Medrano H, Ribas-Carbo M. 2007. Rapid variations of mesophyll conductance in response to changes in CO₂ concentration around leaves. *Plant, Cell & Environment* **30**: 1284-1298.

Flint SD, Ryel RJ, Caldwell MM. 2003. Ecosystem UV-B experiments in terrestrial communities: a review of recent findings and methodologies. *Agriculture Forest Metrology* **120**: 177-189.

Fredeen AL, Randerson JT, Holbrook NM, Field CB. 1997. Elevated atmospheric CO₂ increases water availability in a water limited grassland ecosystem. *Journal of American Water Resource Association* **33**: 1033–1039.

Freeland WJ, Janzen DH. 1974. Strategies in herbivory by mammals: The Role of Plant Secondary Compounds. *American Naturalist* **108**: 269-281.

Friso G, Spetea C, Giacometti GM, Vass I, Barbato R. 1994a. Degradation of Photosystem II reaction centre D1 protein induced by UVB radiation in isolated thylakoids. Identification and characterization of C- and N-terminal breakdown products. *Biochimica et Biophysica Acta* **1194**: 78–84.

Friso G, Barbato R, Giacometti GM, Barber J. 1994b. Degradation of D2 protein due to UV-B irradiation of the reaction centre of Photosystem II. *FEBS Letters* **339**: 217–221.

Galmés J, Medrano H, Flexas J. 2006. Acclimation of Rubisco specificity factor to drought in tobacco: discrepancies between in vitro and in vivo estimations. *Journal of Experimental Botany* **57**: 3659–3667.

Galmés J, Abadia A, Cifre J, Medrano H, Flexas J. 2007. Photoprotection processes under water stress and recovery in Mediterranean plants with different growth forms and leaf habitats. *Physiologia Plantarum* **130**: 495-510.

Genty B, Briantais JM, Baker N. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* **990**: 87–92.

Gerten D, Luo Y, Le Marie G, Parton WJ, Keough C, Weng E, Beier C, Ciais P, Cramer W, Dukes JS, Hanson PJ, Knapp AKK, Linders S, Nepstand D, Rustad L, Sowerby A. 2008. Modelled effects of precipitation on ecosystem carbon and water dynamics in different climatic zones. *Global Change Biology* **14**: 2365-2379.

Gifford RM. 1992. Interaction of carbon dioxide with growth-limiting environmental factors in vegetation productivity: implications for the global carbon cycle. *Advances in Bioclimatology* **1**: 24–58.

Gill RA, Jackson RB. 2000. Global patterns of root turnover for terrestrial ecosystems. *New Phytologist* **147**: 13-31.

Giménez C, Mitchell VJ, Lawlor DW. 1992. Regulation of photosynthesis rate of two sunflower hybrids under water stress. *Plant Physiology* **98**: 516–524.

de Graaff MA, van Groenigen KJ, Six J, Hungate B, van Kessel C. 2006. Interactions between plant growth and soil nutrient cycling under elevated CO₂. *Global Change Biology* **12**: 2077-2091.

Gimingham CH. 1972. Ecology of Heathlands. Chapman and Hall, London 1972: 266pp.

Gordon C, Woodin SJ, Mullins CE, Alexander IJ. 1999a. Effects of environmental change, including drought, on water use by competing *Calluna vulgaris* (heather) and *Pteridium aquilinum* (bracken). *Functional Ecology* **13**: 96-106.

Gordon C, Woodin SJ, Alexander IJ, Mullins CE. 1999b. Effects of increased temperature, drought and nitrogen supply on two upland perennials of contrasting functional type: *Calluna vulgaris* and *Pteridium aquilinum*. *New Phytologist* **142**: 243-258.

Graglia E, Jonasson S, Michelsen A, Schmidt IK, Havsrröm M, Gustavsson L. 2001. Effects of environmental perturbations on abundance of subarctic plants after three, seven and ten years of treatments. *Ecography* **24**: 5-12.

Grassi G, Magnani F. 2005. Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant, Cell and Environment* **28**: 834–849.

Greenberg BM, Wilson MI, Gerhardt KE, Wilson KE. 1996. Morphological and physiological responses of *Brassica napus* to ultraviolet-B radiation: Photomodification of ribulose-1,5- bisphosphate carboxylase/oxygenase and potential acclimation processes. *Journal of Plant Physiology* **148**: 78–85.

Grime JP, Hodgson JG, Hunt R. 1988. Comparative plant ecology: a functional approach to common British species. London, United Kingdom, Unwin Hyman.

Gröbner JA, Albold M, Blumthaler T, Cabot A, De la Casiniere, Lenoble J, Martin T, Masserot D, Müller M, Philipona R, Pichler T, Pougatch E, Rengarajan G, Schmucki D, Seckmeyer G, Sergeant C, Toure ML, Weihs P. 2000. Variability of spectral solar ultraviolet irradiance in an alpine environment. *Journal of Geophysical Research* **105**: 26991-27003.

Gunasekera D, Berkowitz GA. 1993. Use of transgenic plants with Rubisco antisense DNA to evaluate the rate limitation of photosynthesis under water stress. *Plant Physiology* **103**: 629–35.

Gwynn-Jones D, Lee JA, Callaghan TV. 1997. Effects of enhanced UV-B radiation and elevated carbon dioxide concentration on sub-Arctic forest heath system. *Plant Ecology* **128**:242-249.

Haapala JK, Mörsky SK, Saarino S, Rinnan R, Suokanerva H, Kyrö E, Latola K, Martikainen PJ, Holopainen T, Jouko S. 2009. Carbon dioxide balance of a fen ecosystem in northern Finland under elevated UV-B radiation. *Global Change Biology* **15**: 943-954.

Hamerlynck EP, McAllister CA, Knapp AK, Ham JM, Owensby CE. 1997. Photosynthetic gas exchange and water relation responses of three tallgrass prairie species to elevated carbon dioxide and moderate drought. *International Journal of Plant Science* **158**: 608–616.

Harper CW, Blair JM, Fay PA, Knapp AK, Carlisle JD. 2005. Increased rainfall variability and reduced rainfall amount decreases soil CO₂ flux in a grassland ecosystem. *Global Change Biology* **11**: 322–334.

Harley PC, Loreto F, Di Marco G, Sharkey TD. 1992. Theoretical considerations when estimating the mesophyll conductance to CO₂ flux by the analysis of the response of photosynthesis to CO₂. *Plant Physiology* **98**: 1429–1436.

Harte J, Shaw R. 1995. Shifting dominance within a montane vegetation community: results of a climate warming experiment. *Science* **267**: 876-880.

Havaux M, Canaani O, Malkin S. 1987. Inhibition of photosynthetic activities under slow water stress measured in vivo by the photoacoustic method. *Physiologia Plantarum* **70**: 503–510.

He J, Huang L-K, Chow WS, Whitecross MI, Anderson JM. 1993. Effects of supplementary ultraviolet-B radiation on rice and pea plants. *Australian Journal of Plant Physiology* **20**: 129–142.

Henry HAL, Juarez JD, Field CB, Vitousek PM. 2005. Interactive effects of elevated CO₂, N deposition and climate change on extracellular enzyme activity and soil density fractionation in a California annual grassland. *Global Change Biology* **11**: 1808 - 1815

Heimann M, Reichstein M. 2008. Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature* **451**: 289-292.

Heisler JA, Weltzin JF. 2006. Variability matters: Towards a perspective on the influence of precipitation on terrestrial ecosystems. *New Phytologist* **172**: 189-192.

Hobbie SE. 1996. Temperature and plant species control over litter decomposition in Alaskan tundra. *Ecological Monographs* **66**: 503-522.

Hungate BA, Holland EA, Jackson RB, Chapin III FS, Mooney HA, Field CB. 1997. The fate of carbon in grasslands under carbon dioxide enrichment. *Nature* **388**: 576-579.

Hungate BA, Johnson DW, Dijkstra P, Hymus G, Stiling P, Megonigal JP, Pagel AL, Moan JL, Day F, Li J, Hinkle CR, Drake BG. 2006. Nitrogen cycling during seven years of atmospheric CO₂ enrichment in a scrub oak woodland. *Ecology* **87**: 26-40.

Høye TT, Post E, Meltøfte H, Schmidt NM, Forchhammer MC. 2007. Rapid advancement of spring in the High Arctic. *Current Biology* **17**: 449-451.

Iason GR, Hester AJ. 1993. The response of Heather (*Calluna Vulgaris*) to Shade and Nutrients--Predictions of the Carbon-Nutrient balance hypothesis. *The Journal of Ecology* **81**: 75-80.

IPCC. 2001. Climatic change 2001: The Scientific Basis. Intergovernmental Panel on Climate Change, New York, Cambridge University Press.

IPCC. 2007. Climatic change 2007: The Physical Science Basis. Intergovernmental Panel on Climate Change, Geneva.

Jackson GE, Irvine J, Grace J. 1999. Xylem acoustic emissions and water relations of *Calluna vulgaris* L. at two climatological regions of Britain. *Plant Ecology* **140**: 3-14.

Jacobs CMJ, de Bruin HAR. 1997. Predicting regional transpiration at elevated atmospheric CO₂: influence of the PBL-vegetation interaction. *Journal of Applied Meteorology* **36**: 1663-1675.

Jansen MAK, Gaba V, Greenberg BM, Mattoo AK, Edelman M. 1996. Low threshold levels of ultraviolet-B in a background of photosynthetically active radiation trigger rapid degradation of the D2 protein of photosystem II. *Plant Journal* **9**: 693-696.

Jahnke S, Krewitt M. 2002. Atmospheric CO₂ concentration may directly affect leaf respiration measurement in tobacco, but not respiration itself. *Plant, Cell and Environment* **25**: 641-651.

Jentsch A, Kreylling J, Boettcher-Treschow J, Beierkuhnlein C. 2008. Beyond gradual warming: extreme weather events alter flower phenology of European grassland and heath species. *Global Change Biology* **14**: 1-13.

Jokela K, Leszczynski K, Visuri R. 1993. Effects of Arctic ozone depletion and snow on UV exposure in Finland. *Photochemistry and Photobiology* **58**: 559-566.

Johanson U, Gehrke C, Björn LO, Callaghan TV. 1995a. The effects of enhanced UV-B radiation on the growth of dwarf shrubs in a Subarctic heathland. *Functional Ecology* **9**: 713-719.

Johanson U, Gehrke C, Björn LO, Callaghan TV, Sonesson M. 1995b. The effects of enhanced UV-B radiation on subarctic ecosystem. *Ambio* **24**: 106-111.

Johnson D, Campbell CD, Lee JA , Callaghan TV, Gwynn-Jones D. 2002. Arctic microorganisms respond more to elevated UV-B radiation than CO₂. *Nature* **416**: 82–83.

Jokela K, Leszczynski K, Visuri R. 1993. Effects of arctic ozone depletion and snow on UV-B exposure in Finland. *Photochemistry and Photobiology* **58**: 559-518.

Jordan BR, He J, Chow WS, Anderson JM. 1992. Changes in mRNA levels and polypeptide subunits of ribulose 1,5 biphosphate carboxylase in response to supplementary ultraviolet-B radiation. *Plant, Cell & Environment* **15**: 91–98.

Jones HG. 1973. Moderate term water stress and associated changes in some photosynthetic parameters in cotton. *New Phytologist* **97**: 970-974.

Karnosky DF, Mankovska B, Percy K, Dickson RE, Podila GK, Sôber J, Noormets A, Hendrey G, Coleman MD, Kubiske M, Pregitzer KS, Isebrands JG. 1999. Effects of tropospheric O₃ on trembling aspen and interaction with CO₂: Results from an O₃-gradient and a FACE experiment. *Water, Air and Soil Pollution* **116**: 311-322.

Kerr RA. 1993. The ozone reaches a new low. *Science* **262**: 1032-1034.

Kelliher FM, Leuning R, Schulze ED. 1993. Evaporation and canopy characteristics of coniferous forests and grasslands. *Oecologia* **95**: 153–163.

Keiller DR, Holmes MG. 2001. Effects of long term exposure to elevated UV-B radiation on the photosynthetic performance of five broad leaved tree species. *Photosynthesis Research* **67**: 229-240.

Keiller DR, Mackerness SA-H, Holmes MG. 2003. The action of a range of supplementary ultraviolet (UV) wavelengths on photosynthesis in *Brassica napus* L. in the natural environment: effects on PSII, CO₂ assimilation and level of chloroplast proteins. *Photosynthesis Research* **75**: 139-150.

Kimball BA, Idso SB, Johnson S, Rillig MC. 2007. Seventeen years of carbondioxide enrichment of sour orange trees: final results. *Global Change Biology* **12**: 2171-2183.

King JS, Pregitzer KS, Zak DR, Sober J, Isebrands JG, Dickson RE, Hendrey GR, Karnosky DF. 2001. Fine-root biomass and fluxes of soil carbon in young stands of paper birch and trembling aspen as affected by elevated atmospheric CO₂ and tropospheric O₃. *Oecologia* **128**: 237-250.

Kirschbaum MUF. 1987. Water-stress in *Eucalyptus pauciflora* – comparison of effects on stomatal conductance with effects on the mesophyll capacity for photosynthesis, and investigation of a possible involvement of photoinhibition. *Planta* **171**: 466–473.

Kirschbaum MUF. 1988. Recovery of photosynthesis from water stress in *Eucalyptus pauciflora* – a process in two stages. *Plant, Cell and Environment* **11**: 685–694.

Knapp AK, Hamerlynck EP, Ham JM, Owensby CE. 1996. Responses in stomatal conductance to elevated CO₂ in 12 grassland species that differ in growth form. *Vegetatio* **125**: 31–41.

Knapp AK, Briggs JM, Koelliker JK. 2001. Frequency and extent of water limitation to primary production in a mesic temperate grassland. *Ecosystems* **4**: 19-28.

Knapp KA, Fay PA, Blair JM, Collins SL, Smidth MD, Carlisle JD, Harper CW, Danner BT, Lett MS, McCarron JK. 2002. Rainfall variability, carbon cycling, and Plant species diversity in a mesic grassland. *Science* **298**: 2202-2205.

Koch GW, Mooney HA. 1996. Response of terrestrial ecosystems to elevated CO₂: a synthesis and summary. *In*: Koch GW, Mooney HA (eds) Carbon dioxide and terrestrial ecosystems. Academic Press, San Diego, pp 415–429

Kozaki A, Takeba G. 1996. Photorespiration protects C₃ plants from photooxidation. *Nature* **384**: 557–560.

Krause GH, Jahns P. 2004. Non-photochemical energy dissipation determined by chlorophyll-a fluorescence quenching: Characterization and function. *In*: Papageorgiou GC, Govindjee (eds.) Chlorophyll a Fluorescence: A Signature of Photosynthesis. *Advances in photosynthesis and respiration* Volume **19**. Germany, Berlin: Springer, 463-495.

Körner C. 2000. Biosphere responses to CO₂ enrichment. *Ecological Applications* **10**: 1590–1619.

Körner C. 2003. Carbon limitation in trees. *Journal of Ecology* **91**: 4-17.

Körner C, Asshoff R, Bignucolo O, Hättenschwiler S, Keel SG, Peláez-Riedl S, Pepin S, Siegwolf RTW, Zotz G. 2005. Carbon flux and growth in mature deciduous forest trees exposed to elevated CO₂. *Science* **309**: 1360 – 1362.

Körner C. 2006. Plant CO₂ responses: An issue of definition, time and resource supply. *New Phytologist* **172**: 393-411.

Lauber IR, Körner C. 1997, *In situ* stomatal response to long term CO₂ enrichment in calcareous grassland plants. *Acta Oecologia* **18**: 221-229.

Lawlor DW. 1995. The effects of water deficit on photosynthesis. *In*: Smirnoff N (ed.), *Environment and Plant Metabolism. Flexibility and Acclimation*. Oxford, UK: BIOS Scientific, pp129–160.

Lawlor DW. 2002. Limitation of photosynthesis in water-stressed leaves: Stomata vs. metabolism and the role of ATP. *Annals of Botany* **89**: 871-885.

Lawlor DW, Cornic G. 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant, Cell and Environment* **25**: 275–294.

Lehrner G, Delatorre J, Lütz C, Cardemil L. 2001. Field studies on the photosynthesis of two desert Chilean plants: *Prosopis chilensis* and *Prosopis tamarugo*. *Journal of Photochemistry and Photobiology B. Biology* **64**: 36-44.

Leuzinger S, Körner C. 2007. Water savings in mature deciduous forest trees under elevated CO₂. *Global Change Biology* **13**: 2498–2508.

Liberloo M, Tulva I, Raim O, Kull O, Ceulemans R. 2007. Photosynthetic stimulation under long-term CO₂ enrichment and fertilization is sustained across a closed *Populus* canopy profile (EUROFACE). *New Phytologist* **173**: 537-549.

Llorens L, Penuelas J, Beier C, Emmett BA, Estiarte M, Tietema A. 2004. Effects of an experimental increase of temperature and drought in the photosynthetic performance of two ericaceous shrub species along a north–south European gradient. *Ecosystems* **7**: 613–624.

Long SP, Drake BG. 1992. Photosynthetic CO₂ assimilation and rising CO₂ concentrations. *In*: *Crop photosynthesis spatial and temporal determinants* (Baker NR, Thomas H, eds.), Elsevier, Amsterdam, The Netherlands, pp69-103.

Long SP, Bernacchi CJ. 2003. Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *Journal of Experimental Botany* **54**: 2393–2401.

Long SP, Ainsworth EA, Rogers A, Ort DR. 2004. Rising atmospheric carbon dioxide: plants FACE the future. *Annual review of Plant Biology* **55**: 591-628.

Long SP, Ainsworth EA, Bernacchi EA, Davey PA, Hymus GJ, Leahey ADB, Morgan PB, Osborne CP. 2006. Long term responses of photosynthesis and stomata to elevated CO₂ in managed systems. In *Managed Ecosystems and CO₂. Case studies, Processes and Perspectives* (eds Nösberger J, Long SP, Norby RJ, Stitt M, Hendry GR, Blum H.), Springer-Verlag, Heidelberg, Germany, pp 253-270,

Long SP, Ainsworth EA, Leahey ABD, Nosberger J, Ort DR. 2006b. Food for thought: lower than expected crop yield stimulation with rising CO₂ concentrations. *Science* **312**: 1918-1921.

Loreto F, Harley PC, Di Marco G, Sharkey TD. 1992. Estimation of mesophyll conductance to CO₂ flux by three different methods. *Plant Physiology* **98**: 1437–1443.

Lou Y, Wan S, Hui D, Wallace LL. 2001. Acclimatization of soil respiration to warming in a tall grass prairie. *Nature* **413**: 622-625.

Lou Y, Su B, Currie WS, Dukes JS, Finzi A, Hartwig U, Hungate B, McMurtrie RE, Oreon R, Parton WJ, Pataki DE, Shaw MR, Zak DR, Field CB. 2004. Progressive Nitrogen Limitation of Ecosystem Responses to Rising Atmospheric Carbon Dioxide. *Bioscience* **54**: 731-739.

Lou Y. 2007. Terrestrial carbon cycle feedback to climate warming. *Annual Review of Ecology, Evolution and Systematics* **38**: 683-712.

Luo Y, Gerten D, Le Marie G, Parton WJ, Weng E, Zhou X, Keough C, Beier C, Ciais P, Cramer W, Dukes JS, Emmett B, Hanson PJ, Knapp AKK, Linders S, Nepstand D, Rustad L,. 2008. Modeled interactive effects of precipitation, temperature, and [CO₂] on ecosystem carbon and water dynamics in different climatic zones
Global Change Biology **14**: 1986-1999.

MacDonald NW, Zak DR, Pregitzer KS. 1995. Temperature effects on kinetics of microbial respiration and net nitrogen and sulfur mineralization. *Soil Science Society of America Journal* **59**: 233-240.

Madronic S, McKencie RL, Björn LO, Caldwell MM. 1998. Changes in biologically ultraviolet radiation reaching the Earth's surface. *Journal of Photochemistry and Photobiology B. Biology* **46**: 5-19.

Malhi SS, McGrill WB, Nyborg N. 1990. Nitrate losses in soils: effects of temperature, moisture, and substrate concentration. *Soil Biology and Biochemistry* **22**: 917-927.

MacKenzie R, Connor B, Bodeker G. 1999. Increased summertime UV radiation in New Zealand in response to ozone loss. *Science* **285**: 1709-1711.

Manter DK, Kerrigan J. 2004. A/C_i curve analysis across a range of woody plant species: influence of regression analysis parameters and mesophyll conductance. *Journal of Experimental Botany* **55**: 2581–2588.

Margoils H, Oren R, Whitehead D, Kaufman M. 1995. Leaf area dynamics of conifer forests. In: Smith W.K., Hinckley T.M. (eds.) *Ecophysiology of coniferous forests*. Academic Press. San Diego, London. Pp 181-223.

Mattoo AK, Giardi MT, Raskind A, Edelman M. 1999. Dynamic metabolism of Photosystem II reaction center proteins and pigments. *Physiologia Plantarum* **107**: 454–461.

Mencuccini M, Grace J. 1995. Climate influence the leaf area-sapwood area relation ship in Scots pine (*Pinus sylvestris* L.) *Tree Physiology* **15**: 1-10.

Melis A, Nemson JA, Harrison MA. 1992. Damage to functional components and partial degradation of Photosystem II reaction centre proteins upon chloroplast exposure to ultraviolet-B radiation. *Biochimica et Biophysica Acta* **1109**: 312-320.

Medlyn BE, Barton CVM, Broadmeadow MSJ, Ceuleman R, De Angelis P, Forstreuter M, Freeman M, Jackson SB, Kellomaki S, Laitat E, Rey A, Roberntz P, Sigurdsson BD, Strassemeier J, Wang K, Curtis PS, Jarvis PG. 2001. Stomatal conductance of forest species after long-term exposure to elevated CO₂ concentration: a synthesis. *New Phytologist* **149**: 247–264.

Medrano H, Parry MA, Socias X, Lawlor DW. 1997. Long term water stress inactivates Rubisco in subterranean clover. *Annals of Applied Biology* **131**: 491–501.

Medrano H., Escalona JM, Bota J, Gulias J, Flexas J. 2002. Regulation of photosynthesis of C₃ plants in response to progressive drought: Stomatal conductance as a reference parameter. *Annals of Botany* **89**: 895-905.

Meentemeyer V. 1978. Macroclimate and lignin control of litter decomposition rates. *Ecology* **59**: 465-472.

Menzel A, Fabian P. 1999. Growing season extended in Europe. *Nature* **397**: 659.

Menzel A, Sparks TH, Estrella N, Koch E, Aasa A, Ahas R, Alm-Kûbler K, Bissolli P, Braslavska O, Briede A, Chielewski FM, Crepensek Z, Curnel Y, Dahl Å, Defila C, Donnelly A, Filella Y, Jatzczak K, Måge F, Mestre A, Nordli Ø, Penuelas J, Pirinen P, Remosova V, Scheifinger H, Striz M, Susnik A, van

Vliet AJH, Wielgolaski F-E, Zach S, Zust A. 2006. European phenological response to climate change matches the warming pattern. *Global Change Biology* **12**: 1969-1976.

Meyer S, de Kouchkovsky Y. 1992. ATPase state and activity in thylakoids from normal and water-stressed Lupin. *FEBS Letters* **303**: 233–236.

Michelsen A, Graglia E, Schmidt IK, Jonasson S, Sleep D, Quarmby C. 1999. Differential grass and dwarf shrub responses to long term changes in soil microbial biomass C, N, and P by factorial NPK fertilizer fungicide and labile carbon addition to a heath. *New Phytologist* **143**: 523-538.

Middleton EM, Teramura AH. 1993. The protective role of flavonol glycosides and carotenoids in protecting soyabean from UV-B damage. *Plant Physiology* **103**: 741-752.

Miglietta F, Peressotti A, Vaccari FP, Zaldei A, deAngelis P, Scarascia-Mugnozza G. 2001. Free-air CO₂ enrichment (FACE) of a poplar plantation: the POPFACE fumigation system. *New Phytologist* **150**: 465–476.

Mikkelsen TN, Beier C, Jonasson S, Holmstrup M, Schmidt IK, Ambus P, Pilegaard K, Michelsen A, Albert KR, Andresen LC, Arndal MF, Bruun N, Christensen S, Danbæk S, Gundersen P, Jørgensen P, Kongstad J, Maraldo K, Primé A, Riis-Nielsen T, Ro-Poulsen H, Stevnbak K, Selsted MB, Sørensen P, Larsen KS, Carter MS, Martinussen T, Miglietta F, Sverdrup H. 2008. Experimental design of multifactor climate change experiments with elevated CO₂, warming and drought – the CLIMATE project. *Functional Ecology* **22**: 185-195.

Miyashita K, Tanakamaru S, Maitani T, Kimura K. 2005. Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. *Environmental and Experimental Botany* **53**: 205–214.

Moore BD, Cheng S-H, Sims D, Seemann JR. 1999. The biochemical and molecular basis for photosynthetic acclimation to elevated atmospheric CO₂. *Plant Cell and Environment* **22**: 567-582.

Morgan JA, Pataki DE, Körner C, Clark H, Del Grosso SJ, Grünzweig JM, Knapp AK, Mosier AR, Newton PCD, Niklaus PA, Nippert JB, Nowak RS, Parton WJ, Polley HW, Shaw MR. 2004. Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO₂. *Oecologia* **140**: 11–25.

Morison JIL, Gifford RM. (1984) Plant growth and water use with limited water supply in high CO₂ concentrations. II. Plant dry weight, partitioning and water use efficiency. *Australian Journal of Plant Physiology* **11**: 375–384.

Morgan JA, Pataki DE, Körner C, Clark H, Del Grosso SJ, Grünzweig JM, Knapp AK, Mosier AR, Newton PCD., Niklaus PA, Nippert JB, Nowak RS, Parton WJ, Polley HW, Shaw MR. 2004. Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO₂. *Oecologia* **140**: 11–25.

Murthy R, Barron-Gafford G, Dougherty PM, Engel VC, Grieve K, Handley L, Klimas C, Potosnak MJ, Zarnoch SJ, Zhang JW. 2005. Increased leaf area dominates carbon flux response to elevated CO₂ in stands of *Populus deltoides* (Bartr.). *Global Change Biology* **11**: 716–731.

Negash L, Björn LO. 1986. Stomatal closure by ultraviolet radiation. *Physiologia Plantarum* **66**: 360-364.

Niklaus PA, Spinnler D, Körner C. 1998. Soil moisture dynamics of calcareous grassland under elevated CO₂. *Oecologia* **117**: 201–208.

Norby RJ, Lou Y. 2004. Evaluating ecosystem responses to rising atmospheric CO₂ and global warming in a multi-factor world. *New Phytologist* **162**: 281-293.

Norby RJ, DeLucia EH, Gielen B Jr. 2005. Forest response to elevated CO₂ is conserved across a broad range of productivity. *Proceedings of the National Academy of Science* **102**: 18052-18056.

Nogués S, Baker NR. 1995. Evaluation of the role of damage to Photosystem II in the inhibition of CO₂ assimilation in pea leaves on exposure to UV-B radiation. *Plant, Cell & Environment* **18**: 781–787.

Nowak RS, Ellsworth DS, Smith SD. 2004. Functional responses of plants to elevated atmospheric CO₂ – do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytologist* **162**: 253-280.

Obrist D, Verburg PSJ, Young MH, Coleman JS, Schorran DE, Arnone JA. 2003. Quantifying the effects of phenology on ecosystem evapotranspiration in planted grassland mesocosms using EcoCELL technology. *Agriculture, Forest Meteorology* **118**: 173–183.

Oren R, Ellsworth DS, Johnsen KH, Phillips N, Ewers BE, Maier C, Schäfer KVR, McCarthy H, Hendry G, McNulty SG, Katul GC. 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO₂ enriched atmosphere. *Nature* **411**: 469-472.

Parry MAJ, Andralojc PJ, Mitchell RAC, Madgwick PJ, Keys AJ. 2003. Manipulation of Rubisco: the amount, activity, function and regulation. *Journal of Experimental Botany* **54**:1321-1333.

Paul MJ, Foyer CH. 2001. Sink regulation of photosynthesis. *Journal of Experimental Botany* **360**: 1383-1400.

Paul N. 2001. Plant responses to UV-B: time to look beyond stratospheric ozone depletion? *New Phytologist* **150**: 1-8.

Penuelas J, Filella I. 2001. Responses to a warming world. *Science* **294**: 793-795.

Peñuelas J, Gordon C, Llorens L, Nielsen T, Tietema A, Beier C, Bruna P, Emmett B, Estiarte M, Gorissen A. 2004. Nonintrusive field experiments show different plant responses to warming and drought among sites, seasons, and species in a North–South European gradient. *Ecosystems* **7**: 598-621

Peñuelas J, Prieto P, Beier C, Cesaraccio C, de Angelis P, de Dato G, Emmett BA, Estiarte M, Garadnai J, Gorissen A, Lang EK, Kröel-Dulay G, Llorens L, Pellizzaro G, Riis-Nielsen T, Schmidt IK, Sirca C, Sowerby A, Spano D, Tietema A. 2007. Response of plant species richness and primary productivity in shrublands along a north-south gradient in Europe to seven years of experimental warming and drought: reductions in primary productivity in the heat and drought year of 2003. *Global Change Biology* **13**: 2563-2581.

Peterjohn WT, Adams MB, Gilliam FS. 1994. Symptoms of nitrogen saturation in two central Appalachian hardwood forest ecosystems. *Biogeochemistry* **35**: 507-522.

Phillips DL, Johnson MG, Tingey DT, Catricala CE, Hoyman TL, Nowak RS. 2006. Effects of elevated CO₂ on fine root dynamics in a Mojave Desert community: a FACE study. *Global Change Biology* **12**: 61–73.

Phoenix GK, Gwynn-Jones D, Lee JA, Callaghan TV. 2000. The impacts of UV-B radiation on the regeneration of a sub-arctic heath community. *Plant Ecology* **146**: 67-75.

Phoenix GK, Gwynn-Jones D, Callaghan TV, Sleep D, Lee JA. 2001. Effects of global change on a sub-arctic heath: effects of enhanced levels of UV-B radiation and increased summer precipitation. *Journal of Ecology* **89**: 256–267.

Phoenix GK, Gwynn-Jones D, Lee JA, Callaghan TV. 2002. Ecological importance of solar ultraviolet radiation to a sub-arctic heath community. *Plant Ecology* **165**: 263-273.

Phoenix GK, Lee JA. 2004. Predicting impacts of Arctic climate change: Past lessons and future challenges. *Ecological Research* **19**: 65-74.

Piao S, Friedlingstein P, Ciais P, Viovy N, Demarty J. 2007. Growing season extension and its impact on terrestrial carbon cycle in the Northern Hemisphere over the past 2 decades. *Global Biogeochemical Cycles* **21**: GB3018.

Pons TL, Welschen RAM. 2002. Overestimation of respiration rates in commercially available clamp-on leaf chambers. Complications with measurement of net photosynthesis. *Plant, Cell & Environment* **25**: 1367–1372.

Pregitzer KS, King JS, Burton AJ, Brown SE. 2000. Responses of tree fine roots to temperature. *New Phytologist* **147**: 105-115.

Raich JW, Schlesinger WH. 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus* **44**: 81-89.

Reichstein M, Tenhunen JD, Rouspard O, Ourcival J-M, Rambal S, Miglietta F, Peressotti A, Pecchiari M, Tirone G, Valentini R. 2002. Severe drought effects on ecosystem CO₂ and H₂O fluxes at three Mediterranean evergreen sites: revision of current hypotheses? *Global change Biology* **8**: 999-1017.

Reich PB, Hobbie SE, Lee T, Ellsworth DS, West JB, Tilman D, Knops JMH, Naeem S, Trost J. 2006. Nitrogen limitation constrains sustainability of ecosystem response to CO₂. *Nature* **440**: 922-925.

Rinnan R, Keinänen MM, Kasurinen A, Asikainen J, Kekki TK, Holopainen T, Ro-Poulsen H, Mikkelsen TN, Michelsen A. 2005. Ambient UV radiation in the arctic reduces root biomass and alter microbial community composition but has no effect on microbial biomass. *Global Change Biology* **11**: 564-574.

Robberecht R, Caldwell MM, Billings WD. 1980. Leaf ultraviolet optical properties along a latitudinal gradient in the arctic-alpine lifezone. *Ecology* **61**: 612-619.

Robson TM, Pancotto VA, Flint SD, Balleré CL, Sala OE, Scopel AL, Caldwell MM. 2003. Six years of solar UV-B manipulations affect growth of *Sphagnum* and vascular plants in a Tierra del Fuego peatland. *New Phytologist* **160**: 379-389.

Robredo A, Pérez-López U, Sainz de la Maza H, González-Moro B, Lacuesta M, Mena-Petite A, Munoz-Rueda A. 2007. Elevated CO₂ alleviates the impact of drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis. *Environmental and Experimental Botany* **59**: 252-263.

Rouspard O, Gross P, Dreyer E. 1996. Limitation of photosynthetic activity by CO₂ availability in the chloroplasts of oak leaves from different species and during drought. *Annales des Sciences Forestieres* **53**: 243–254

Rousseaux MC, Balleré CL, Giordano CV, Scopel AL, Zima AM, Szwarcberg-Bracchitta M, Searles PS, Caldwell MM, Diaz SB. 1999. Ozone depletion and UV-B radiation: Impact on DNA damage in southern South America. *Proceedings of the national Academy of Sciences of the USA* **96**: 15310-15315.

Rousseaux MC, Flint SD, Searles PS, Caldwell MM. 2004. Plant responses to current solar UV-B radiation and to supplemented solar ultraviolet-B radiation simulating ozone depletion: An experimental comparison. *Journal of Photochemistry and Photobiology B Biology* **80**: 224-230.

Rogers A, Fischer BU, Bryant J, Frehner M, Blum H, Raines CA, Long SP. 1998. Acclimation of photosynthesis to elevated CO₂ under low-nitrogen nutrition is affected by the capacity for assimilate utilization. Perennial ryegrass under free air CO₂ enrichment. *Plant Physiology* **118**: 683-689.

Rogers A, Allen DJ, Davey PA, Morgan PB, Ainsworth EA, Bernacchi CJ, Cornic G, Dermody O, Dohleman FG, Heaton EA, Mahoney J, Zhu G, Delucia EH, Ort DR, Long SP. 2004. Leaf photosynthesis and carbohydrate dynamics of soybeans grown throughout their life-cycle under Free-Air Carbon dioxide enrichment. *Plant Cell and Environment* **27**: 449-458.

Rogers A, Ainsworth EA. 2006. The response of foliar carbohydrates to elevated carbon dioxide concentration. *In: Managed Ecosystems and CO₂. Case Studies, Processes and Perspectives* (eds. Nösberger J, Long SP, Norby RJ, Stitt M, Hendrey GR, Blum H), Springer Verlag, Heidelberg, Germany, pp293-308.

Rogers A, Ainsworth EA, Kamman C. 2006. Perspectives of the future of free air CO₂ enrichment studies. *In: Managed Ecosystems and CO₂. Case studies, Processes and Perspectives* (eds Nösberger J., Long S.P., Norby R.J., Stitte M., Hendrey G.H., Blum H.), Springer-Verlag, Heidelberg, Germany, pp431-450.

Rozema J, Boelen P, Blokker P. 2005. Depletion of stratospheric ozone over Antarctic and Arctic: Responses of plants of polar terrestrial ecosystems to enhanced UV-B, an overview. *Environmental Pollution* **137**: 428-442.

Rozema J, Boelen P, Solheim B, Zielke M, Buskens A, Doorenbosch M, Fijn R, Herder J, Callaghan T, Björn LO, Gwynn-Jones D, Broekman R, Blokker P, van de Poll W. 2006. Stratospheric ozone depletion: High arctic tundra plant growth on Svalbard is not affected by enhanced UV-B after 7 years of UV-B supplementation in the field. *Plant Ecology* **182**: 121–135.

Ruhland CT, Day TA. 2000. Effects of ultraviolet-B radiation on leaf elongation, production and phenylpropanoid concentrations of *Deschampsia antarctica* and *Colobanthus quietensis* in Antarctica. *Physiologia Plantarum* **109**: 244-351.

Ruhland CT, Xiong FS, Clark WD, Day TA. 2005. Symposium-in-Print: Ultraviolet radiation and terrestrial ecosystems the influence of ultraviolet-B radiation on growth, hydroxycinnamic acids and flavonoids of *Deschampsia antarctica* during springtime

ozone depletion in Antarctica. *Photochemistry and Photobiology* **81**: 1086–1093.

Rustad L, Campbell J, Marion G, Norby R, Mitchell M, Hartley A, Cornelissen J, Gurevitch J, GCTE-NEWS. 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* **126**: 543–562.

Rustad LE. 2006. From transient to steady state response of ecosystems to atmospheric CO₂-enrichment and global climate change: Conceptual challenges and need for an integrated research. *Plant Ecology* **182**: 43-62.

Rustad LE. 2008. The response of terrestrial ecosystems to global climate change: Towards an integrated approach. *Science of the total environment* **404**: 222-235.

Sage RF, Kubien DS. 2007. The temperature response of C3 and C4 photosynthesis. *Plant, Cell and Environment* **30**: 1086-1106.

Schansker G, Toth SZ, Strasser RJ. 2006. Dark recovery of the Chl a fluorescence transient (OJIP) after light adaptation: The qT-component of non-photochemical quenching is related to an activated photosystem I acceptor side. *Biochimica et Biophysica Acta –Bioenergetics* **1757**: 787-797.

Schmidt IK, Beier C, Kongstad J, Andresen L, Michelsen A, Albert KR, Ambus P, Selsted M, Maraldo K, Holmstrup M, Arndal M, Mikkelsen TN, Ro-Poulsen H, Jonassen S, Karsten RJ. 2007. Klimæændringer og processer og funktion i terrestriske økosystemer. *Flora og Fauna* **113**: 117-128.

Schindelbacher A. Zechmeister-Boltenstern S, Jandl R. 2009. Carbon losses due to soil warming: Do autotrophic and heterotrophic soil respiration respond equally? *Global Change Biology* **15**: 901-913.

Schulze E-D. 1986. Carbon dioxide and water vapor exchange in response to drought in the atmosphere and soil. *Annual review of Plant Physiology* **37**: 247-274.

Scurfield G. 1954. Biological Flora of the British Isles, *Deschampsia flexuosa* (L.) Trin. *Journal of Ecology* **42**: 225–233.

Searles PS, Flint SD, Caldwell MM. 2001. A meta-analysis of plant field studies simulating stratospheric ozone depletion. *Oecologia* **127**: 1–10.

Schreiber U. 2004. Pulse-Amplitude-Modulation (PAM) fluorimetry and saturation pulse method: an overview. In: Papageogiou GC, Govindjee (eds.) *Advances in Photosynthesis and Respiration*. Berlin, Germany: Springer, pp279-319.

Shaver GR, Canadell J, Chapin FS III, Gurevitch J, Harte J, Henry G, Ineson P, Jonasson S, Melillo J, Pitelka L, Rustad L. 2000. Global warming and terrestrial ecosystems: a conceptual framework for analysis. *Bioscience* **50**: 871-882.

Shaw MR, Zavaleta ES, Chiariello NR, Cleland EE, Mooney HA, Field CB. 2002. Grassland responses to global environmental changes suppressed by elevated CO₂. *Science* **298**: 1987-1990.

Sharkey TD. 1985. Photosynthesis in intact leaves of C₃ plants: physics, physiology and rate limitations. *The Botanical Review* **51**: 53-105.

Shindell DT, Rind D, Lonergan P. 1998. Increased polar stratospheric ozone losses and delayed eventual recovery owing to increasing greenhouse-gas concentration. *Nature* **392**: 589-592.

Solheim B, Johanson U, Callaghan TV, Lee JA, Gwynn-Jones D, Björn LO. 2002. The nitrogen fixation potential of arctic cryptogam species is influenced by enhanced UV-B radiation. *Oecologia* **133**: 90-93.

Springer JC, Ward JK. 2007. Flowering time and CO₂. *New Phytologist* **176**: 243-255.

Strasser RT, Srivastava A, Govindjee. 1995. Polyphasic chlorophyll-a fluorescence transients in plants and cyanobacteria. *Journal of Photochemistry and Photobiology B* **61**: 32-42.

Strasser RJ, Tsimilli-Michael M, Srivastava A. 2004. Analysis of the chlorophyll-a fluorescence transient. In: Papageogiou GC, Govindjee (eds.) *Advances in Photosynthesis and Respiration*. Berlin, Germany: Springer, pp321-362.

Strid A, Chow WS, Anderson JM. 1990. Effects of supplementary ultraviolet-B radiation on photosynthesis in *Pisum sativum*. *Biochimica et Biophysica Acta* **1020**: 260-268.

Srivastava A, Guissé B, Greppin H, Strasser RT. 1997. Regulation of antenna structure and electron transport in photosystem II of *Pisum sativum* under elevated temperature probed by the fast polyphasic fluorescence transients: OKJIP. *Biochimica et Biophysica Acta* **1320**: 95-106.

Taalas P, Kaurola J, Kylling A. 2000. The impact of greenhouse gases and halogenated species on the future solar UV radiation doses. *Geophysical Research Letters* **27**: 1127-1130.

Telfer A, Barber J. 1994. Elucidating the molecular mechanisms of photoinhibition by studying isolated Photosystem II reaction centres. *In*: Baker NR, Bowyer JR, eds. Author Environmental Plant Biology Series; Photoinhibition of photosynthesis: From molecular mechanisms to the field, Oxford, United Kingdom, BIOS Scientific Publishers, pp25-49.

Teng N, Wang J, Chen T, Wu X, Wang Y, Lin J. 2006. Elevated CO₂ induces physiological, biochemical and structural changes in leaves of *Arabidopsis thaliana*. *New Phytologist* **172**: 378-378.

Terashima I, Wong SC, Osmond CB, Farquhar GD. 1988. Characterization of non-uniform photosynthesis induced by abscisic acid in leaves having different mesophyll anatomies. *Plant and Cell Physiology* **29**: 385–394.

Terashima I, Ono K. 2002. Effects of HgCl₂ on CO₂ dependence of leaf photosynthesis: evidence indicating involvement of aquaporins in CO₂ diffusion across the plasma membrane. *Plant and Cell Physiology* **43**: 70–78.

Tezara W, Mitchell VJ, Driscoll SD, Lawlor DW. 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature* **401**: 914–917.

Teramura AH, Tevini M, Iwanzik W. 1983. Effects of ultraviolet-B irradiance on plants during a mild winter stress. I. Effects of diurnal stomatal resistance. *Physiologia Plantarum* **57**: 175-180.

Teramura AH, Sullivan JH. 1994. Effects of UV-B radiation on photosynthesis and growth of terrestrial plants. *Photosynthesis Research* **39**: 463-473.

Tevini M. 2004. Plant Responses to Ultraviolet Radiation Stress. *In*: Papageorgiou GC, Govindjee eds. Chlorophyll a Fluorescence: A Signature of Photosynthesis. *Advances in photosynthesis and respiration* Volume 19. Dordrecht, Netherland, pp605-621.

Turnbull MH, Murthy R, Griffin KL. 2002. The relative impacts of daytime and night time warming on photosynthetic capacity in *Populus deltoides*. *Plant Cell and Environment* **25**: 1729-1737.

Turnbull MH, Tissue DT, Murthy R, Wang X, Sparrow AD, Griffin KL. 2004. Nocturnal warming increases photosynthesis at elevated CO₂ partial pressure in *Populus deltoides*. *New Phytologist* **161**: 819-826.

Tyree MT, Wilmot TR. 1990. Errors in the calculation of evaporation and leaf conductance in steady-state porometry—the importance of accurate measurement of leaf temperature. *Canadian Journal of Forest Research* **20**: 1031–1035.

Valentini R, Matteucci G, Dolman AJ, Schulze E-D, Rebmann C, Moors EJ, Granier A, Gross P, Jensen NO, Pilegaard K, Lindroth A, Grelle A, Bernhofer C, Grünwald T, Aubinet M, Ceulemans R, Kowalski AS, Vesala T, Rannik Ü, Berbigier P, Loustau D, Gumundsson J, Thorgeirsson H, Ibrom A, Morgenstern K, Clement R, Moncrieff J, Montagnani L, Minerbi S, Jarvis P. G. 17. 2000. Respiration as the main determinant of carbon balance in European forests. *Nature* **404**: 861–865.

Verhoef A. 1997. The effect of temperature differences between porometer head and leaf surface on stomatal conductance measurements. *Plant, Cell & Environment* **20**: 641–646.

Volk M, Niklaus PA, Körner C. 2000. Soil moisture effects determine CO₂ responses of grassland species *Oecologia* **125**: 380–388.

van Heerden PDR, Tsimilli-Michael M, Krüger GHJ, Strasser RJ. 2003. Dark chilling effects on soybean genotypes during vegetative development: Parallel studies of CO₂ assimilation, chlorophyll-a fluorescence kinetics O-J-I-P and nitrogen fixation. *Physiologia Plantarum* **117**: 476–491.

Vu CV, Allen DJ, Garrard LA. 1984. Effects of enhanced UV-B radiation (280 – 320 nanometers) on ribulose 1,5-bisphosphate carboxylation in pea [*Pisum sativum* cultivar Little Marvel] and soybean [*Glycine max* cultivar Bragg]. *Environmental and Experimental Botany* **24**: 131–144.

Walker MD, Wahren CH, Hollister RD, Henry GHR, Ahlquist LE, Alatalo JM, Bret-Harte MS, Calef MP, Callaghan TV, Carroll AB, Epstein HE, Jonsdottir IS, Klein JA, Magnusson B, Molau U, Oberbauer SF, Rewa SP, Robinson CH, Shaver GR, Suding KN, Thompson CC, Tolvanen A, Totland Ø, Turner PL, Tweedie CE, Webber PJ, Wookey PA. 2006. Plant community responses to experimental warming across the tundra biome. *Proceedings of the National Academy of Sciences* **31**: 1342–1346.

Wall GW, Brooks TJ, Adam NR, Cousins A, Triggs J, Kimball BA, Pinter PJ Jr, LaMorte RL, Ottman MJ, Leavitt SW, Matthias AD, Williams DG, Webber AN. 2001. Leaf photosynthesis and water relations of grain sorghum grown in free-air CO₂ enrichment (FACE) and water stress. *New Phytologist* **152**: 231–248

Walther G-R, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, Fromentin J-M, Hoegh-Guldberg O, Bairlein F. 2002. Ecological responses to recent climate change. *Nature* **416**: 389–395.

Wan S, Luo Y, Wallace LL. 2002. Changes in microclimate induced by experimental warming and clipping in tallgrass prairie. *Global Change Biology* **8**: 754–768.

Wan S, Hui D, Wallace L, Lou Y. 2005. Direct and indirect effects of experimental warming on ecosystem carbon processes in a tall grass prairie. *Global Biochemical Cycles* **19**: GB2014. Doi:10.1029/2004GB002315.

Ward SJE, Midgley GF, Jones MH, Curtis PS. 1999. Responses of wild C4 and C3 grass (Poaceae) species to elevated atmospheric CO₂ concentration: a meta-analytic test of current theories and perceptions. *Global Change Biology* **5**: 723–741.

Warren Wilson J. 1957. An analysis of plant growth and its control in arctic environments. *Annals of Botany* **30**: 383–402.

Webb AR. 1997. Monitoring changes in UV-B radiation. In: Lumsden PJ (ed.) *Plants and UV-B: Responses to environmental change*. Cambridge University Press, Cambridge United Kingdom, pp 13–30. ISBN 0-521-57222-3

Welker JM, Brown KB, Fahnestock JT. 1999. CO₂ flux in arctic and alpine dry tundra: comparative field responses under ambient and experimentally warmed conditions. *Arctic and Antarctic Alpine Research* **31**: 272–277.

Welker JM, Fahnestock JT, Jones MH. 2000. Annual CO₂ flux from dry and moist arctic tundra: field responses to increases in summer temperature and winter snow depth. *Climatic Change* **44**: 139–150.

Weathershead B, Tanskanen A, Stevermer A. 2005. Ozone and Ultraviolet radiation. In: Symon C, Arris L, Heal B, (eds.) *ACIA, Arctic Climate Impact Assessment*, New York, USA: Cambridge University Press, pp151–183.

White MA, Running SW, Thornton PE. 1999. The impact of growing-season length variability on carbon assimilation and evapotranspiration over 88 years in the eastern US deciduous forest. *International Journal of Biometeorology* **42**:139–145.

WMO 2003. Scientific Assessment of Ozone Depletion: 2002. Global Ozone Research and Monitoring Project. Report no. 47. World Meteorological Organization, Geneva, Switzerland, 498 pp

Xiong FS, Day TA. 2001. Effect of Solar Ultraviolet-B Radiation during Springtime Ozone Depletion on Photosynthesis and Biomass Production of Antarctic Vascular Plants. *Plant Physiology* **125**: 738–751.

Yordanov I. Velikova V, Tsonev T. 2000. Plant responses to drought, acclimation, and stress tolerance. *Photosynthetica* **38**: 171-186.

Zavaleta ES, Thomas BD, Chiariello NR, Asner GP, Shaw MR, Field CB. 2003. Plants reverse warming effect on ecosystem water balance. *Proceedings of the National Academy of Science in USA* **100**: 9892–9893.

Appendix:

Publication list for Kristian Rost Albert March 2009

Total: Peer-review papers (6), Submitted papers (4), Abstracts (21), Reports (12).

Peer-Review:

1. **Albert KR**, Ro-Poulsen H, Mikkelsen TN, Bredahl L, Haakanson KB. (2005) Effects of reducing the ambient UV-B radiation in the High Arctic on *Salix arctica* and *Vaccinium uliginosum*. *Phyton* 45: 41-49
2. **Albert KR**, Mikkelsen TN, Ro-Poulsen H. (2005) Effects of ambient versus reduced UV-B radiation on high arctic *Salix arctica* assessed by measurements and calculations of chlorophyll-a fluorescence parameters from fluorescence transients. *Physiologia Plantarum* 124: 208-226
3. Mikkelsen TN, Beier C, Jonasson S, Holmstrup M, Schmidt IK, Ambus P, Pilegaard K, Michelsen A, **Albert KR**, Andresen LC, Arndal MF, Bruun N, Christensen S, Danbæk S, Gundersen P, Jørgensen P, Kongstad J, Maraldo K, Primé A, Riis-Nielsen T, Ro-Poulsen H, Stevnbak K, Selsted MB, Sørensen P, Larsen KS, Carter MS, Martinussen T, Miglietta F, Sverdrup H. (2008) Experimental design of multifactor climate change experiments with elevated CO₂, warming and drought – the CLIMAITE project *Functional Ecology* 22: 185-195
4. **Albert KR**, Rinnan R, Ro-Poulsen H, Mikkelsen TN, Håkansson KB, Arndal MF, Michelsen A. (2008) Solar Ultraviolet-B Radiation (UV-B) at Zackenberg: The Impact on Higher Plants and Soil Microbial Communities *In: Meltofte H, Christensen T, Elberling B, Forchammer M, Rasch M Elsevier, Dynamics of a High Arctic Ecosystem, Advances in Ecological Research* 40: 421-440
5. **Albert KR**, Ro-Poulsen H, Mikkelsen TN. (2008) Ambient UV-B radiation decreases photosynthesis in high arctic *Vaccinium uliginosum* *Physiologia Plantarum* 133: 199-210
6. Arndal MF, Illeris L, Michelsen M, **Albert KR**, Tamstorf M, Hansen BU. (2008) Seasonal variation in gross ecosystem production, standing biomass, C and N pools in five high arctic vegetation types *Arctic and Antarctic Alpine Research: in press*

Submitted Manuscripts:

1. Grøndahl L, Tamstorf M, Friberg T, Soegaard H, Illeris L, Hansen BU, **Albert KR**, Arndal M, Pedersen MR and Michelsen A. (200x) Scaling CO₂ fluxes from plot- and field-level to landscape-level in a high arctic ecosystem using a footprint model and satellite images. *Submitted to Global Change Biology*

2. **Albert KR**, Arndal MF, Boesgaard K, Mikkelsen TN, Michaelsen A, Håkansson KB, Schmidt NM, Ro-Poulsen H. (200x) Ambient UV-radiation decreases PSII performance in spite of increased screening ability via UV-absorbing compounds in *Salix arctica* and *Vaccinium uliginosum* during six years of UV-exclusion in high arctic heaths. *Submitted to Plant Cell & Environment*
3. Illeris L, Tamstorf MP, Michelsen A, Arndal MF, Grøndahl L, **Albert KR**, Hansen BU. (200x) Growing season CO₂ flux and NDVI in five different vegetation types of high arctic Greenland. *Submitted to Environmental and Experimental Biology*
4. **Albert KR**, Mikkelsen TN, Ro-Poulsen H, Arndal MF, Michelsen A. (200x) Ambient UV-B doses decreases PSII performance and net photosynthesis under natural irradiance in high arctic *Salix arctica* heath. *Submitted to Environmental and Experimental Biology*

Reports

1. Mikkelsen TN, Ro-Poulsen H, Bredahl L, **Albert KR** (2003) Effects of UV-B radiation on vegetation in Zackenberg *In*: Rasch, M and Caning, K (eds) Zackenberg Ecological Research Operations, 8th Annual Report, 2002 - Copenhagen, Danish Polar Center, Ministry of Science, Technology and Innovation
2. Ro-Poulsen H, Mikkelsen TN, Bredahl L, **Albert KR**, Håkansson KB, Rinnan R (2004) Effects of UV-B radiation on vegetation in Zackenberg *In*: Rasch, M and Caning, K (eds) Zackenberg Ecological Research Operations, 9th Annual Report, 2003 - Copenhagen, Danish Polar Center, Ministry of Science, Technology and Innovation
3. Ro-Poulsen, H, **Albert, KR**, Mikkelsen, TN (2004) Monitoring af UV-belastningen af vegetationen i Zackenberg Tre års undersøgelser af UV-B effekter på højarktisk vegetation i NØ-Grønland, Zackenberg Opsummerende rapport til Miljøstyrelsen vedr DANCEA-bevilling JnrM 123/000-0212 (2004) 12 p
4. **Albert KR**, Ro-Poulsen H, Mikkelsen TN, Håkansson KB, Bredahl L, Rinnan R (2005) Monitoring effects of ambient UV-radiation on high arctic vegetation *In*: Rasch, M and Caning, K (eds) Zackenberg Ecological Research Operations, 10th Annual Report, 2004 - Copenhagen, Danish Polar Center, Ministry of Science, Technology and Innovation
5. Tamstorf MP, Illeris L, Arndal MF, Rask Pedersen M, Michelsen A, Hansen BU, Grøndahl L, **Albert KR**, König SM (2005) Spectral Calibration of High Arctic Primary Production Estimation (SCHAPPE) *In*: Rasch, M and Caning, K (eds) Zackenberg Ecological Research Operations, 10th Annual Report, 2004 - Copenhagen, Danish Polar Center, Ministry of Science, Technology and Innovation
6. Michelsen A, Ro-Poulsen H, **Albert KR**, Ellebjerg SM, Arndal MF, Tamstorf M, Illeris L, Mikkelsen TN (2006) Effekter af klima- og UV-B manipulationer

- på processer og organismer i høarktiske terrestriske økosystemer - opsummerende rapport til Miljøstyrelsen 2006 16p
7. **Albert KR**, Arndal MF, Michelsen A, Tamstorf MF, Ro-Poulsen H, Mikkelsen TN (2006) Studying effects of current UV-fluxes on high arctic vegetation (UV-B exclusion experiments) *In*: Rasch, M and Caning, K (eds) Zackenberg Ecological Research Operations, 11th Annual Report, 2005 - Copenhagen, Danish Polar Center, Ministry of Science, Technology and Innovation, 2006
 8. Michelsen A, König SM, Illeris L, Arndal MF, **Albert KR**, Tamstorf MP and Ro-Poulsen H (2006) Effects of climate manipulations on processes and organisms in high arctic terrestrial ecosystems *In*: Rasch, M and Caning, K (eds) Zackenberg Ecological Research Operations, 11th Annual Report, 2005 - Copenhagen, Danish Polar Center, Ministry of Science, Technology and Innovation
 9. **Albert KR**, Boesgaard K, Ro-Poulsen H, Mikkelsen TN, Michelsen A, Schmidt NM (2008) Studies of Plant Responses to UV-B radiation *In*: Rasch, M and Caning, K (eds) Zackenberg Ecological Research Operations, 13th Annual Report, 2007 - Copenhagen, Danish Polar Center, Ministry of Science, Technology and Innovation
 10. Schmidt IK, Beier C, Kongstad J, Andresen L, Michelsen A, **Albert KR**, Ambus P, Selsted M, Maraldo K, Holmstrup M, Arndal M, Mikkelsen TN, Ro-Poulsen H, Jonassen S, Karsten, RJ (2007) Klimaændringer og processer og funktion i terrestriske økosystemer *Flora og Fauna* 113: 117-128
 11. **Albert KR**, Arndal MF, Michelsen A, Tamstorf MF, Ro-Poulsen H, Mikkelsen TN (2007) Effects of current UV-fluxes on high arctic vegetation (UV-B exclusion experiments) *In*: Rasch, M and Caning, K (eds) Zackenberg Ecological Research Operations, 12th Annual Report, 2006 - Copenhagen, Danish Polar Center, Ministry of Science, Technology and Innovation
 12. Michelsen A, Ellebjerg S, Arndal MF, Tamstorf M, Schmidt, NM, Illeris L, **Albert KR**, Ro-Poulsen H (2007) Effects of climate manipulations on processes and organisms in high arctic terrestrial ecosystems *In*: Rasch, M and Caning, K (eds) Zackenberg Ecological Research Operations, 12th Annual Report, 2006 - Copenhagen, Danish Polar Center, Ministry of Science, Technology and Innovation

Abstracts

1. **Albert KR**, Ro-Poulsen H, Mikkelsen TN (2005) Ambient UV-radiation decreases photosynthetic performance on High Arctic plants 11th biennial meeting of Arctic Biology March 2005 at University of Copenhagen, Denmark Abstract and poster
2. **Albert KR**, Ro-Poulsen H, Mikkelsen TN (2006) How does prolonged drought affect plant physiological processes? - Focus on the interactions with increased temperature and elevated [CO₂] within the CLIMAITE

project EPRECOT conference May 2006 in Helsingør, Denmark Abstract and poster

3. **Albert KR**, Ro-Poulsen H, Mikkelsen TN, Michelsen A, Beier C, Jonasson S, Pilegård K, Ambus P, Kappel Schmidt I, Holmstrup M, Primé A, Christensen S (2006) The Biological Time Machine - Biological responses to multiple environmental and climatic changes Environment and Stress, Phd symposium at KVL October 2006 Abstract, talk and poster
4. Mikkelsen TN, Beier C, Kappel Schmidt I, Michelsen A, **Albert KR**, Ambus P, Andersen K (2006) Climate - a three factor climate change ecosystem manipulation study: set up and approaches for data analysis Ecological Society of America, ESA, 2006 conference annual meeting, Memphis, Tennessee Abstract
5. Ambus P, Selsted MB, Carter MS, **Albert KR**, Andersson M, Larsen KS, Priemé A, Michelsen A, Mikkelsen TN, Pilegaard K, Beier C (2007) Denitrification and N₂O losses in a heath-land under changing climate conditions 14th and final meeting of the ESF COST 856 Action on denitrification and related aspects: Achievements of COST 856, Uppsala Sweden 5-8 December 2007 Abstract
6. Ambus P, Selsted MB, Carter MS, **Albert KR**, Andersson M, Larsen KS, Michelsen A, Mikkelsen TN, Pilegaard K, Beier C (2007) Combined effects of drought, temperature and CO₂ on GHG emissions from temperate shrub-land COST 639 plenary meeting, Vienna, Switzerland 12-13 Apr 2007 Abstract
7. Mikkelsen TN, Beier C, **Albert KR**, Ro-Poulsen H (2007) CLIMATE – a three factor climate change ecosystem manipulation experiment AGU fall meeting 10-14 december 2007 in San Fransisco, USA Abstract
8. **Albert KR**, Mikkelsen TN, Ro-Poulsen H, Arndal MF, Michelsen A (2008) Ambient UV-B decreases PSII performance and net photosynthesis under natural irradiance of the high arctic dwarf shrub *Salix arctica* Nordic Photosynthetic Congress, 21-24 October 2008, Faculty of Life Sciences, University of Copenhagen, Denmark Abstract, talk and poster
9. Ro-Poulsen H, **Albert KR**, Mikkelsen TN, Michelsen A (2008) Plant eco-physiological response patterns to summer drought, elevated CO₂, and warming in a semi-natural temperate heath ecosystem Nordic Photosynthetic Congress, 21-24 October 2008, Faculty of Life Sciences, University of Copenhagen, Denmark Abstract, talk and poster
10. **Albert KR**, Mikkelsen TN, Ro-Poulsen H, Michelsen A (2008) Plant eco-physiological response patterns to summer drought, elevated CO₂ and warming in a natural temperate heath ecosystem Ecological Society of America, 93rd ESA annual meeting August 3-8, Milwaukee, Wisconsin Abstract and talk
11. **Albert KR**, Rinnan R, Mikkelsen TN, Ro-Poulsen H, Michelsen A, Arndal MF, Schmidt NM (2008) Biological responses to current fluxes of ultraviolet radiation in High Arctic After the Melt - International

- Conference on Ecological Responses to Arctic Climate Change, May 5-7 2008, University of Aarhus, Denmark Abstract and poster
12. **Albert KR**, Mikkelsen TN, Ro-Poulsen H (2008) Biological responses to current UV-B radiation in Arctic regions Geophysical Research Abstracts, Vol 10, EGU2008-A-11698, 2008 SRef-ID: 1607-7962/gra/EGU2008-A-11698 EGU General Assembly 13-18 2008 April Vienna, Switzerland Abstract and poster
 13. Mikkelsen T N, Beier C, Jonasson S, Holmstrup M, Schmidt I K, Ambus P, Pilegaard K, Michelsen A, **Albert KR**, Andresen L C, Arndal M F, Bruun N, Christensen S, Danbæk S, Gundersen P, Jørgensen P, Linden LG, Kongstad J, Maraldo K, Priemé A, Riis-Nielsen T, Ro-Poulsen H, Stevnbak K, Selsted M B, Sørensen P, Larsen K S, Carter M S, Ibrom A, Martinussen T, Miglietta F, Sverdrup H (2008) CLIMAITE – a three factor climate change ecosystem manipulation experiment Geophysical Research Abstracts, Vol 10, EGU2008-A-07931, 2008 SRef-ID: 1607-7962/gra/EGU2008-A-07931 EGU General Assembly 13-18 April 2008 in Vienna, Switzerland Abstract and poster
 14. **Albert KR**, Mikkelsen TN, Ro-Poulsen H (2008) Future photosynthetic performance of heath plants – results from the multi-factorial ecosystem experiment, CLIMAITE 5th SPPS 2008 PhD conference January 24-27 2008 in Haslev, Denmark Abstract, talk and poster
 15. **Albert KR**, Mikkelsen TN, Ro-Poulsen H (2008) Biological responses to current UV-B radiation in Arctic regions 5th SPPS 2008 PhD conference January 24-27 2008 in Haslev, Denmark Abstract and poster
 16. Carter MS, **Albert KR**, Ambus P (2009) is organic farming a mitigation option? – A study on N₂O emission from winter wheat Copenhagen Climate Change Congress 2009. Abstract and poster.
 17. Selsted MB, **Albert KR**, Ambus P, Michelsen A, Ro-Poulsen H, Mikkelsen TN, Ibrom A (2009) Ecosystem Recovery after Drought Events in Our Future Climate Copenhagen Climate Change Congress 2009. Abstract and poster.
 18. Linden LG, Beier C, Mikkelsen TN, Holmstrup M, Schmidt IK, Ambus P, Pilegaard K, **Albert KR**, Andresen LC, Selsted MB, Larsen KS, Ibrom A (2009) Ecosystem carbon balance under future climate conditions: The CLIMAITE project carbon synthesis Copenhagen Climate Change Congress 2009. Abstract and poster.
 19. Ro-Poulsen H, **Albert KR**, Michelsen A, Mikkelsen TN, Beier C (2009) Unpredictable responses to interactions between climatic drivers: Impact of warming, elevated CO₂, drought and their combinations on photosynthesis and growth patterns of heath plants Biogeomon 2009, Helsinki *Accepted for oral presentation*
 20. Selsted MB, **Albert KR**, Ambus P, Michelsen A, Ro-Poulsen H, Mikkelsen TN, Ibrom A (2009) Responses of carbon and water fluxes following drought events in combinations with warming and elevated CO₂ Biogeomon 2009, Helsinki *Accepted for poster session*

21. Carter MS, Ambus P, **Albert KR**, Larsen KS, Andersson M, Andresen LC, Mikkelsen TN, Beier C (2009) N₂O and CH₄ fluxes in a temperate heathland under future climatic conditions NCGG-5 in Wageningen June 2009. *Accepted for poster session*

Paper I

Growth and eco-physiological responses of *Calluna vulgaris* and *Deschampsia flexuosa* to natural drought and warm autumn in a temperate heath

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Manuscript

Growth and eco-physiological responses of *Calluna vulgaris* and *Deschampsia flexuosa* to natural drought and warm autumn in a temperate heath

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Abstract

Primary productivity is affected by plant phenological growth patterns and their responsiveness towards adapting to environmental changes. Insight in these processes is needed to understand primary productivity responses to climatic changes and for accurate up-scaling of ecosystem feedbacks. We investigated the characteristics of photosynthetic performance and phenology in the stress tolerant dwarf scrub *Calluna vulgaris* and the opportunistic grass species *Deschampsia flexuosa* in a temperate heath. We described the seasonal growth patterns by measuring specific leaf weights, leaf carbon, nitrogen and $\delta^{13}\text{C}$ in both species, together with the average green leaf length per shoot in *Deschampsia*, and the shoot green-to-brown ratio in *Calluna*. Photosynthetic performance was characterized monthly during the main growing season by conducting leaf gas-exchange (A/C_i curves) to investigate net photosynthesis, stomatal conductance, intercellular CO_2 concentration and photosynthetic capacity parameters (V_{cmax} , J_{max} and R_d). Primary productivity was limited by periodically low water supply and when the evergreen *Calluna* endured severe water stress the above ground biomass of *Deschampsia* wilted. Rewetting increased primary productivity immediately in *Calluna* whereas *Deschampsia* grew new leaves. The primary productivity of both species benefitted from the extraordinary warm autumn temperatures. These responses suggest a pronounced potential for further primary productivity stimulation in response to autumn warming, increased precipitation during summer, or to elevated CO_2 .

Introduction

The CO₂ concentration, precipitation patterns, temperature and nitrogen deposition influence in concert plant eco-physiological processes (Rustad 2008, Heimann and Reichstein 2008, Lou *et al.* 2008) and much effort is currently put into understanding these responses (Penuelas *et al.* 2007, Prieto *et al.* 2009, Mänd *et al.* 2009). To predict plant responses to climate change, it is important to study both the direct effects of elevated temperature, atmospheric CO₂ concentrations and altered precipitation patterns on plant processes but also to gain knowledge on plant characteristics and their seasonal variability, as seasonality is expected to change with climatic changes. Warming leads to earlier onset of the growing season (Cleland *et al.* 2006, Penuelas *et al.* 2003, Prieto *et al.* 2009, Menzel *et al.* 2006, Wan *et al.* 2004) and potentially increased carbon uptake (White *et al.* 1999, Piao *et al.* 2007). However, warming may also stimulate soil microbes that decompose litter, returning a comparable amount of CO₂ to the atmosphere (Piao *et al.* 2007). In addition, precipitation patterns are expected to change, resulting in more extreme events, such as periodic drought or flooding (Houghton *et al.* 2001), but also seasonal changes are expected as prolonged summer drought and higher precipitation during winter is expected in Denmark. The impact of precipitation variability and frequency are of importance for productivity (Knapp *et al.* 2002, Heisler and Weltzin 2006) and severe drought events can substantially decrease productivity (Ciais *et al.* 2005). These changes will all impact eco-physiological processes, with effect on primary productivity. More knowledge on these processes is therefore needed for development of predictive models on ecosystem feedbacks to climate change (Rustad 2008, Heimann and Reichstein 2008).

Heather is a slow growing evergreen shrub classed as a stress tolerant competitor (Grime *et al.* 1988) having adaptive traits for dry and nutrient low habitats e.g. xeromorphic leaves, long leaf life span, low nitrogen content, low nutrient loss and likely low photosynthetic rates (Aerts 1995). Short term acclimation to experimental drought decreased the plant water potential (Jackson *et al.* 1995), decreased the light saturated net photosynthesis, transpiration and stomatal conductance in *Calluna*, but had little or no effect on WUE (Gordon *et al.* 1999a, 1999b). Across an European gradient precipitation were a major factor controlling the magnitude of *Calluna* photosynthesis (Llorens *et al.* 2004). In the long term a decreased ratio of leaf area to sapwood reduced transpirational water loss in *Calluna* on sites differing with low soil water availability (Jackson *et al.* 1999), in line with responses in other woody plants (Margoils *et al.* 1995, Mencuccini and Grace 1995). Shoot winter browning (Watson *et al.* 1966, Braid and Tervet 1937) has been suggested to be an effect of low water potential and low soil moisture (Jackson *et al.* 1999). Warming advanced phenology in *Calluna* (Jentsch 2008) but the responses of *Calluna* to warming depend on soil water availability (Penuelas *et al.* 2007).

Grasses often have opportunistic growth patterns with rapid leaf growth, high leaf nitrogen content (Grime *et al.* 1988) and likely high photosynthetic rates. The grass *Deschampsia*, which dominates in North European heaths, has rhizomes (Scurfield 1954) and a maximal leaf life span less than a year, but there are always some green leaves, giving *Deschampsia* a 'semi-evergreen' character (Aerts 1993). In addition, *Deschampsia* has a variable number of leaf cohorts per shoot which likely allow a large degree of flexibility to initiate or extend growth in response to environmental changes. While a number of studies are available on *Calluna* growth patterns (Aerts 1989, Aerts 1993,

Barclay-Estrup 1970, Gimingham 1972), less information is available on *Deschampsia*, the most important grass species in Danish heathlands. However, a number of studies on *Deschampsia* have focused on heathland conservation and the effects of nutrient additions, e.g. on regeneration in competition with other species (Britton *et al.* 2003, Aerts *et al.* 1990, Aerts and Chapin 2000, Alonso and Hartley 1998) and vegetative responses to changes in PAR (Foggo and Warrington 1989, Foggo 1989). However, no study has characterized the photosynthetic performance of *Deschampsia*, its seasonal variability and responses to environmental changes. We therefore investigated detailed the seasonal variation in plant eco-physiology and growth patterns of *Calluna vulgaris* and *Deschampsia flexuosa*. We studied the light saturated net photosynthesis, stomatal conductance and leaf ^{13}C , an integrated measure of water use efficiency over time (Farquhar, Ehlinger and Hubrick 1989). Furthermore, we investigated changes in plant cover, leaf length and concentration of carbon and nitrogen in leaves. To our knowledge the leaf gas-exchange studies provide the first information available on photosynthetic capacity, J_{max} , V_{cmax} and r_d in these dominant heath plants, from the onset of the growing season to senescence in fall.

We hypothesized firstly that the two plant species would perform differently to the natural drought in late summer, with the most pronounced negative impact on photosynthetic performance on the evergreen dwarf shrub, and the most pronounced negative impact on growth on the grass. Secondly, *Calluna* was as an evergreen expected to be able to make more use of an extended warm autumn.

Materials and Methods

Site characteristics

The study was carried out at the heathland Brandbjerg (55°53'N 11°58'E) about 50 km NW of Copenhagen, Denmark. It is a dry heathland ecosystem with the evergreen dwarf scrub *Calluna vulgaris* (L.) and the C3 grass *Deschampsia flexuosa* (L.) as the dominant plant species. The soil is a nutrient-poor sandy moraine from the Weichsel glaciations and the N deposition at the site is ca. 1.3 g N/m²/year (Larsen *et al.*, unpublished). The investigations were made in the control plots of the CLIMAITE experiment (Mikkelsen *et al.* 2008), where the meteorological and soil data were collected.

Leaf growth

Growth of *Deschampsia* and *Calluna* was studied in six replicated plots. In each plot, 4 random shoots of *Deschampsia flexuosa* were tagged in March 2005 before the onset of the growing season. The total and the green leaf lengths of each leaf were measured with a ruler from a fixed point at the transition between leaf blade and sheath (Jonasson and Chapin 1985). The measurements were repeated with two weeks intervals from March to October 2005. To investigate the difference in phenological leaf growth the shoots were as soon as distinguishable divided into two groups; flowering and non-flowering (vegetative) shoots.

Cover and biomass

Two permanently marked 0.5 x 0.5 m subplots for vegetation analysis were placed in each of the six replicates. We used the non-destructive pin-point analysis (Jonasson 1988,

Graglia *et al.* 2001) where a frame with a 10 x 10 cm fixed grid pattern was placed above the vegetation. A 2 mm diameter pin was lowered vertically into the vegetation at the 25 points in the grid. Each hit on plant parts by the pin was registered by species and height until the tip of the pin was no longer visible. Further it was recorded if the pin hit newly dead or alive plant parts. This procedure was repeated at each grid point. A pin-point analysis provides estimates of plant cover defined as the relative number of pins in the grid that touch the species. The analyses were done twice in the growth season, June and August 2006. The frame was placed exactly in the same position for each analysis. The point frequency method has shown to be correlated to plant biomass (Jonasson 1988) and here we used a site specific calibration where biomass was estimated from the number of pin hits for *Deschampsia* and a combination of maximum height and cover for *Calluna* (Riis-Nielsen and Schmidt, unpublished).

Leaf gas exchange

Measurements were conducted within the control plots at the CLIMAITE site.

Gas exchange measurements of CO₂ and H₂O fluxes were conducted *in situ* on *Deschampsia flexuosa* and *Calluna vulgaris* by using two LI-6400's (LI-COR Biosciences, Lincoln, Nebraska, USA) connected to standard 2x3 cm² chambers with LED light source (6400-02B). The measurements were carried out monthly in 2006: May (11-18th); June (12-16th); July (10-13th); August (14-21st); September (11-22nd) and October (9-20th). Only healthy shoots and leaves from the top of the vegetation were selected. Pilot studies resulted in following the methodologies securing highly reproducible measurements: 1) *Calluna* shoots from the top of the canopy were flattened

between nylon strings 0.2 mm thick suspended in a metal frame with spacing on 2.5-4 mm. The position of the shoots was upright and approximated to face south. 2) *Deschampsia* leaves, ca. 10-20, were positioned side by side and gently fixed by hairpins during the measurement. Leaf cuvettes were then fixed to the samples using flexible tripods (Magic Arm 143, Manfrotto, Italy). The leaf material was harvested after measurements and care was taken to ensure that weight and area were determined on exactly the same material as inserted in the leaf cuvette.

The protocol for [CO₂] response curves were optimized and tested. Samples were acclimated 2-4 minutes at ambient CO₂ (380 ppm), until net photosynthesis and stomatal conductance were stabilized (± 1 CV over 30 sec). The CO₂ response curves were measured by stepping down [CO₂] (from 380 ppm to 300, 200, 100, 50 ppm) and then taken back to 380 ppm for 4 minutes re-acclimation until initial state was reached again. Then [CO₂] was stepped up to saturation (from 380 ppm to 450, 510, 650, 800, 1000, 1200, 1400 ppm) at the saturating light level 1500 $\mu\text{mol photons/m}^2/\text{s}$, using the LI-COR Auto program 'A/Ci-curve' (Settings: Min 45 and max 60 sec, Reference CO₂ stable in 10 sec with CV<1%, C_i stable in 10 sec with CV<1%, IRGA matching performed at each step). Block temperature was held constant at 25°C and sample relative humidity were stabilized at 45-55% during measurements. All measurements were area corrected. Calculation of maximum velocity of Rubisco carboxylation, V_{cmax} and maximum velocity of Rubisco regeneration, J_{max}, followed the approach of Dubois *et al.* (2007) based on the principles originating from Farquhar *et al.* (1980). Gas-exchange parameters as the light saturated photosynthesis, P_n, transpiration, T_r, stomatal conductance, g_s, intercellular CO₂

concentration, C_i , were extracted from the CO_2 response curves at reference $\text{CO}_2 = 380$ ppm CO_2 .

Leaf biomass, area and chemistry

Immediately after harvest digital pictures were taken of plant material flattened by transparent Plexiglas beside a brick of known area. The photographs were converted to black and white and loaded in a pixel counting program (Bitmap, S. Danbæk, Department of Biology, University of Copenhagen) and the leaf areas were determined. Then the fresh weight of *Calluna* and *Deschampsia* were determined. The harvested *Calluna* shoots were split in to photosynthetic active parts and the rest, i.e. 'green' and 'brown' fractions, respectively. The dry weight was determined after oven drying at 80°C. The leaf material was analyzed for C and N concentration and ^{13}C natural abundance with a Eurovector CN analyzer coupled to an Isoprime isotope ratio mass spectrometer. During analysis, the internal reference gas was calibrated against certified standards from the International Atomic Energy Agency, and plant material calibrated against certified standards was used as working standard. As a measure of current year growth, the green-to-brown ratios were calculated from the dry weights of *Calluna*. Based on dry weights and leaf area of the 'green' fraction of *Calluna*, we calculated the specific leaf weight, SLW.

Statistics

Analyses of variance were made in SAS using the proc mixed to test for effects of the fixed factor and also covariates. Pre-treatment data were initially included in the full

model as covariate and handled as a fixed factor and the significant dependents from the regression analysis were later included as covariates (see below). Significance levels are reported as a tendency with † when $p < 0.10$ and as significant with * when $p < 0.05$, ** when $p < 0.001$ and *** when $p < 0.0001$. All data were normal distributed and some parameters were transformed to fulfill the assumption of variance homogeneity. To interpret the significant responses we compared the least squared means and observed if these were of same magnitude and in the same direction as numerical means. Regression analysis included both photosynthetic and climatic parameters using SAS proc reg. The independent variables were included and kept at the 0.05 level (SLENTY=0.05 and SLSTAY=0.05) to the model optimized by exchanging parameters until only the most significant remained (option STEPWISE). Pearson correlations coefficients were calculated using SAS proc corr.

Results

Climate

The mean daily air temperatures increased above the freezing point in mid to late March, and April was the first month with positive mean temperature, 6.1°C. The snow cover disappeared around early April. Temperature gradually increased into a August mean of 21.3°C and hereafter decreased slowly without any frost events during September (17.3°C) and October (12.9°C) (Fig 1).

Precipitation and soil water are shown in Fig. 1. The mean soil water content (SWC) in 0-20 cm decreased from 13.9 vol% in May to 11.6 vol% in June and 8.8 vol% in July.

Rewetting restored SWC to 15.8 vol% in August, 13.6 vol% in September and 16.5 vol% in October.

Seasonal growth pattern of *Calluna*

Following winter the overwintering shoots of *Calluna* were greening. The top shoots were very compact with many leaves concentrated on short twigs in May and June where after shoots elongated in July. The individual leaves increased leaf area with a faster rate than dry weight as the SLW decreased significantly during May, June and July (Fig 3). The C/N ratio increased from 23.1 in May to a seasonal high of 37.3 in July, mainly because the nitrogen content decreased, from 2.1%DW in May to 1.3%DW in July, and with only marginal change in leaf carbon concentration (Fig. 3). The $\delta^{13}\text{C}$ increased from May to June and July closely reflecting the gradually decreasing soil water content (Fig. 2). The green-to-brown ratio decreased from a similar level in May and June (4.20 ± 0.68 , 4.51 ± 0.76) to a lower level in July (2.9 ± 0.3), in part due to the pronounced elongation of the top shoots but likely also as a response to the decrease in SWC during June-July. The *Calluna* plant cover increased in-significantly from 48.7 ± 9.4 % in June to 55.3 ± 10.0 % in August and the estimated *Calluna* biomass increased from 391 ± 65 g/m³ in June to 474 ± 78 g/m³ in August. In August most *Calluna* plants were in the phase of flowering and a burst of nitrogen was allocated to the shoots (data not shown). Incidents of precipitation likely stimulated growth and nitrogen uptake by the plants, and new shoots grew from the top of the flowering shoots in the end of the flowering period in September. The nitrogen content level in leaves in August, September and October was high, in parallel to the low C/N ratio (Fig. 3). During September and October the C/N

ratio was stable (Fig. 3), and there was marginally increase in the green-to brown ratio (August 0.71 ± 0.07 , September 0.86 ± 0.29 , October 0.88 ± 0.14) and SLW (Fig 3). In August, September and October the values of the $\delta^{13}\text{C}$ closely reflected the SWC, with low values after rewetting increased has the soil water content (Fig 2).

Seasonal growth pattern of *Deschampsia*

In our study only a few short grass leaves of *Deschampsia* overwintered with a March low in cover and biomass (data not shown). During the winter months most of the grass leaves presumably die back with re-translocation from leaves to roots and rhizomes. The grass has a sequential leaf growth and the number of leaf cohorts varied between 3-10 per shoots, either vegetative or flowering (pers.obs). The total green leaf length per shoot (Fig. 5) is the sum of these cohort leaf lengths. The stable SLW (Fig. 3) indicates that the leaf area and the leaf biomass increased with similar rates and therefore the above ground primary production predominantly was allocated to leaf length increases. This means that the total green leaf length per shoot is a good measure of the above ground productivity.

The total green leaf length per vegetative shoot followed two growth phases, with maximum length in late June just before flowering, and a second after flowering with a peak in late August (Fig. 5). Flowering shoots had a major peak in early May and a smaller one in beginning of October and growth rates were generally lower than vegetative shoots (Fig. 5). The first growth phase for the vegetative shoots was initiated in late March to early April, and the mean leaf length per shoot increased from 136 mm on 31 March to a peak of 181 mm on 26 June. This is equal to growth rates up to 3 mm per day coinciding with pre-flowering. From late June mortality resulted in negative

growth rates with a mean leaf length of 126 mm on the 24 July. In the second growth phase late July-August, the growth rates increased up to nearly 3 mm to peak leaf lengths of 181 mm on the 30 August. Growth rates were low in September and became negative in October (Fig. 5). The total green leaf length per flowering shoot had a peak around 1st May before flowering. The negative growth rates during the flowering period suggests that reallocation from these leaves somehow fueled the energy demand for flowering and fruiting. A second build-up started in late July with a maximum around 1st October (Fig. 5).

The first phase dieback of vegetative shoots was likely induced by the natural drought in 2006. The $\delta^{13}\text{C}$ increased from May to July, as a possible response to water shortage, leveled off until August, and decreased from September to October (Fig. 2). The *Deschampsia* plant cover decreased from 65.7 ± 4.8 % in June to 56.3 ± 8.8 % in August, but the estimated green biomass did not change from June to August (119 ± 11 g m⁻² and 122 ± 23 g m⁻² respectively). However, the dead biomass, likely from first phase growth, increased from 126 ± 13 g m⁻² in June to 190 ± 28 g m⁻² in August. First phase leaves were characterized by the nitrogen decrease from May to July while second phase leaves had high nitrogen levels in August, September and October (Fig. 3). As the carbon content was stable around 40-42% during the whole season (Fig. 3) the C/N ratio followed nitrogen the changes in concentration and increased from May to July, after which it decreased to a seasonal low of 16.4 in October (Fig. 3). In 2005, senescence and second phase die-back was initiated in late September/early October with decrease in nutrient concentrations (data not shown).

Leaf gas-exchange

Calluna net photosynthesis decreased significantly from May to the minimum in June and July, increased towards August and September and peaks in October (Fig. 2). Stomatal conductance showed pronounced variation with a decrease from May to seasonal low levels in June and July. Thereafter g_s increased to a high level in August, decreased to a medium in September and was high in October. The same midsummer depression was seen for the intercellular CO_2 concentration (Figure 2). There was no effect of month on photosynthesis when including stomatal conductance ($p < 0.0001$) and intercellular CO_2 concentration ($p < 0.0001$) as covariates. The growing season mean of V_{cmax} was 53.3 ± 11.1 , J_{max} was 80.3 ± 10.8 and R_d 4.0 ± 0.9 (Fig. 4). Like P_n the J_{max} and R_d were highest in October. There was a significant correlation (Pearson correlation coefficient) between net photosynthesis and the following dependents: Stomatal conductance ($p < 0.0001$, $r = 0.43$), intercellular CO_2 ($p < 0.0001$, $r = -0.34$), leaf nitrogen ($p < 0.0001$, $r = 0.33$), SWC in 0-20 cm ($p < 0.0001$, $r = 0.27$) and SWC in 0-60 cm ($p < 0.0001$, $r = 0.21$). Also there was a significant correlation between stomatal conductance and SWC in 0-20 cm ($p < 0.0001$, $r = 0.46$) and SWC in 0-60 cm ($p < 0.0001$, $r = 0.37$). Regression analysis including photosynthetic and climatic parameters revealed that 84% of the variation in net photosynthesis was explained by the parameters stomatal conductance (58%), intercellular CO_2 concentration (12)% and temperature in 0 cm (11%).

Deschampsia net photosynthesis followed about the same pattern as *Calluna* during spring and summer. Across season the net photosynthesis was about 30 % higher per leaf area compared to *Calluna*, but in October it was lower in the grass (Fig. 2).

Stomatal conductance decreased from May to a minimum in June and July whereafter it increased to a seasonal high in August and medium to high values in September and October (Fig. 2). The intercellular CO₂ concentration was at a lower level in May, June and July compared to a higher level in August, September and October (Fig. 2). There was no effect of month on photosynthesis when including stomatal conductance ($p<0.0007$) and intercellular CO₂ concentration ($p<0.0006$) as covariates.

There was a significant correlation between net photosynthesis and stomatal conductance ($p<0.0001$, $r=0.73$), intercellular CO₂ ($p<0.0001$, $r=-0.10$), leaf nitrogen ($p<0.0001$, $r=0.35$), SWC in 0-20 cm ($p<0.0001$, $r=0.37$) and SWC in 0-60 cm ($p<0.0001$, $r=0.23$), and there was a significant correlation between stomatal conductance and firstly, SWC in 0-20 cm, and secondly ($p<0.0001$, $r=0.54$) and SWC in 0-60 cm and ($p<0.0001$, $r=0.45$).

Regression analysis revealed that 69% of the variation in *Deschampsia* net photosynthesis was explained by the parameters stomatal conductance (40%) and intercellular CO₂ concentration (29%).

The growing season mean of J_{\max} and R_d was 96.1 ± 12.8 and 4.1 ± 0.6 , respectively, both highest in July-August (Fig. 4). The V_{\max} level was high in June (74 ± 11) and July (90 ± 12) and low in August, September and October (62 ± 5 , 47 ± 8 , 52 ± 5) with a seasonal mean of 63.8 ± 8.7 (Fig. 4). Regression analysis revealed 89 % of the variation in V_{\max} to be explained by the parameters J_{\max} (26%), nitrogen (24%), PAR (21%), r_d (18 %).

Discussion

The soil water content in 0-20 cm where the major part of the *Calluna* and *Deschampsia* roots are distributed (Arndal, unpublished) decreased from April to near the wilting point in late May due to low precipitation and high plant demand. Interrupted by a rewetting in June, the SWC stayed low during the summer, until the rewetting in August, September and October restored SWC to above May level (Fig. 1). Both plant species responded to low SWC by decreasing stomatal conductance, which over time accumulated into increased $\delta^{13}\text{C}$ indicating water limitation. This behavior reversed after rewetting from August and onwards.

Primary production was not accompanied by a balanced leaf nitrogen uptake in May, June and July as the C/N-ratio increased in *Deschampsia* and even more pronounced in *Calluna*. This pattern with dilution growth of tissue nitrogen contrasts later response where the new leaves of *Calluna* and *Deschampsia* were formed in August, September and October. These leaves had high nitrogen content and the N supply kept pace with growth. These responses are most likely linked to water relations with the sub-optimal soil water content during summer drought likely leading to decreased plant nitrogen uptake while more optimal soil water conditions likely increased the mineralization processes.

Despite the xeromorphic leaves *Calluna* in our study seemed to be affected the most, compared to *Deschampsia*, by the drought probably due to different strategies by those plants. While *Deschampsia* reduced its green biomass during the drought, which reduces the water use per area and enabled high activity in the remaining green leaves. *Calluna* maintained its plant cover and reduced the activity of all leaves.

The photosynthetic capacity in *Calluna* determined by J_{\max} and V_{\max} was remarkably constant during season. In *Deschampsia* the V_{\max} was lower in the new second growth phase leaves, but with no effect on photosynthesis rate. This suggests that the photosynthesis in *Calluna* and *Deschampsia* is regulated mainly by stomatal conductance, which was highly correlated with the photosynthesis. For instance, the *Deschampsia* photosynthesis in May, June and July demonstrate this as here the decreasing stomatal conductance resulted in decreasing intercellular CO_2 concentration and net photosynthesis. In response to rewetting in August, September and October these responses were reversed.

The impact of stomatal conductance may not be surprising since the soil water content decreased to close to the wilting point (about 5%), where stomatal closure were to be expected. At this soil water content we measured plant water potential in the range -3 to -29 bars in *Calluna* shoots (data not shown). This demonstrates the very large range of water supply levels within which *Calluna* is able to operate its stomata in a functional way. The recovery of photosynthesis rates via increased stomatal conductance immediately after precipitation rewetting do further highlight the robustness of *Calluna* being able to cope with soil water content fluctuations. This highlights the stress tolerant strategy adopted by the evergreen *Calluna*. As an evergreen dwarf shrub, it preserves this investment e.g via xeromorphic leaf characteristics. The maintained photosynthesis through the drought period is advantageous when the rewetting begins as *Calluna* then regain productivity immediately using the preserved functional shoots and the high cover per area. This is in contrast to the opportunistic growth strategy by *Deschampsia*, which reduced its green biomass during the natural drought, causing reduced water use per

ground area but fairly high activity in the remaining green leaves. Further *Deschampsia* has showed rapid re-growth when SWC was restored, via initiation of a second phase of leaf growth. This led to a compared to *Calluna*, delayed but high carbon uptake when SWC was not limiting in autumn.

The maximal velocity of Rubisco carboxylation, V_{cmax} , was very stable during growing season in *Calluna*, which indicates the robustness of the photosynthetic capacity in this species. The photosynthetic capacity was more variable in the *Deschampsia*, with two growth phases which produce leaves with different V_{cmax} , lower in the autumn. In both species the photosynthetic rates were correlated with the leaf nitrogen concentration. The high autumn leaf nitrogen concentration in both species leads to their potential for high autumn productivity in response to the favorable environmental conditions, in parallel to high potential for N uptake even under winter conditions (Andresen and Michelsen 2005).

Conclusion

Water availability exerted a large degree of control on the photosynthetic rates in *Calluna* and *Deschampsia*. Both species decreased photosynthesis during drought via decreased stomatal conductance and intercellular CO_2 concentration, but while *Calluna* shoots remained green the *Deschampsia* leaves died back. The two species increased the photosynthetic rates significantly immediately after rewetting, both via increased stomatal conductance and intercellular CO_2 concentration. *Deschampsia* sprouted new leaves and *Calluna* grew new shoots with high nitrogen content and balanced C/N ratio. The similarity of photosynthetic response were in contrast to the different phenological

strategies of the dwarf shrub and the grass. This adds to the need for including phenology when primary productivity patterns are to be understood and up-scaled in models. The unusual high temperature and sufficient water conditions allowed for high photosynthetic activity even in late autumn, extending the growing season.

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References

- Aerts R. (1989)** Above ground nutrient dynamics of *Calluna vulgaris* and *Molinia caerulea* in a dry heathland. *Oikos* **56**: 31-38.
- Aerts R., Berendse F., de Caluwe H., Schmitz M. (1990)** Competition in heathland along an experimental gradient of nutrient availability. *Oikos* **57**: 310-318.
- Aerts R., Chapin III S.F. (2000)** The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research* **30**: 1-67.
- Aerts R. (1993)** Biomass and nutrient dynamics of dominant plant species in heathlands. In: Aerts, R., Heil, G.W. (eds), *Heathlands: Patterns and Processes in a changing environment*. Geobotany, 20, Kluwer Academic Publishers, p51-84.
- Aerts R. (1995)** The advantages of being evergreen. *Tree* **10**: 402-406
- Alonso I., Hartley S.E. (1998)** Effects of nutrient supply, light availability and herbivory on the growth of heather and three competing grasses. *Plant Ecology* **137**: 203-212.
- Andresen LC, Michelsen A. 2005.** Off-season uptake of nitrogen in temperate heath vegetation. *Oecologia* **144**: 585-597.
- Barclay-Estrup, P. (1970)** The description and interpretation of cyclical processes in a heath community. II. Changes in biomass and shoot production during the *Calluna* cycle. *Journal of Ecology* **58**: 243-249.
- Beier C., Emmett B.A., Tietema A., Schmidt I.K., Penuelas J., Lang E.K., Duce P.P., de Angelis P., Gorissen A., Estiarte M., de Dato G., Sowerby A., Gyorgy K., Lellei-Kovacs E., Dyer J., Jones G.E., Kull O., Petersen H., Gjelstrup P., Spano D. (accepted)** Carbon and nitrogen balances for 6 shrublands across Europe. *Global Biochemical Cycles*. *Accepted*.
- Braid K. W., Tervet I.W. (1937)** Certain botanical aspects of the dying-out of heather (*Calluna vulgaris* Hull.). *Scottish Journal of Agriculture* **20**: 1-8.
- Britton A., Marrs R., Pakeman R., Carey P. (2003)** The influence of soil-type, drought and nitrogen addition on interactions between *Calluna vulgaris* and *Deschampsia flexuosa*: implications for heathland regeneration. *Plant Ecology* **166**: 93-105.
- Cleland, E.E., N.R. Chiariello, S.R. Loarie, H.A. Mooney, C.B. Field (2006)** Diverse responses of phenology to global changes in a grassland ecosystem. *Philosophical transactions of the National academy of Science* **103**: 13740-13744.
- Ciais P., Reichstein M., Viovy N., Granier A., Ogée J., Allard V., Aubinet M., Buchmann N., Bernhofer Chr., Carrara A., Chevallier F., De Noblet N., Friend A.**

D., Friedlingstein P., Grünwald T., Heinesch B., Keronen P., Knohl A., Krinner G., Loustau D., Manca G., Matteucci G., Miglietta F., Ourcival J. M., Papale D., Pilegaard K., Rambal S., Seufert G., Soussana J. F., Sanz M. J., Schulze E. D., Vesala T., Valentini R. (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature* **437**: 529-533.

Damgaard C., Riis-Nielsen T., Schmidt I.K. (2009) Estimating plant competition coefficients and predicting community dynamics from non-destructive pin-point data: a case study of *Calluna vulgaris* and *Deschampsia flexuosa*. *Plant Ecology*. in press.

Dubois J.-J.B., Fiscus E.L., Booker F.L., Flowers M.D., Reid C.D. (2007) Optimizing the statistical estimation of the parameters of the Farquhar-von Caemmerer-Berry model of photosynthesis. *New Phytologist* **176**: 402-414.

Farquhar G.D., von Caemmerer S., Berry J.A. (1980) A biochemical model of photosynthetic CO₂ carbon dioxide assimilation in leaves of C₃ carbon pathway species. *Planta* **149**: 78-90.

Farquhar G.D., Ehlinger J.R., Hubrick (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**: 503-537.

Foggo M.N. (1989) Vegetative responses of *Deschampsia flexuosa* (L.) Trin. (Poaceae) seedlings to nitrogen supply and photosynthetically active radiation. *Functional Ecology* **3**: 337-343.

Foggo M.N., Warrington I.J. (1989) Light and vegetative growth in the grasses *Deschampsia flexuosa* (L.) Trin. and *Festuca ovina* L. *Functional Ecology* **3**: 439-445.

Foot J.P., Caporn S.J.M., Lee J.A., Ashenden T.W. (1996) The effect of long term ozone fumigation on the growth, physiology and frost sensitivity of *Calluna vulgaris*. *New Phytologist* **133**: 503-511.

Gimingham C. H. (1972) Ecology of Heathlands. Chapman and Hall, London 1972: 266pp.

Gordon C., Woodin S.J., Mullins C.E., Alexander I.J. (1999a) Effects of environmental change, including drought, on water use by competing *Calluna vulgaris* (heather) and *Pteridium aquilinum* (bracken). *Functional Ecology* **13**: 96-106.

Gordon C., Woodin S.J., Alexander I.J., Mullins C.E. (1999b) Effects of increased temperature, drought and nitrogen supply on two upland perennials of contrasting functional type: *Calluna vulgaris* and *Pteridium aquilinum*. *New Phytologist* **142**: 243-258.

Graglia E., Jonasson S., Michelsen A., Schmidt I. K., Havström M., Gustavsson L. (2001) Effects of environmental perturbations on abundance of subarctic plants after three, seven and ten years of treatments. *Ecography* **24**: 5-12.

Grime J.P., Hodgson J.G., Hunt R. (1988) Comparative plant ecology: a functional approach to common British species. London, United Kingdom, Unwin Hyman.

Heil G., Aerts R. (1993) General introduction. Pages 1-24 in Heathland, Patterns and processes in changing environments. Kluwer academic publishers

Heil G., Bobbink R. (1993) Impact of atmospheric nitrogen deposition on dry heathlands, a stochastic model simulating competition between *Calluna vulgaris* and two grass species. In Aerts R., Heil G. W. (eds.) Heathlands, Patterns and processes in a changing environment. Kluwer academic publishers, Dordrecht.

Heil G.W., Bruggink M. (1987) Competition for nutrients between *Calluna vulgaris* (L.) and *Molinia caerulea* (L.) Moench. *Oecologia* **73**: 105-108.

Heimann M., Reichstein M. (2008) Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature* **451**: 289-292.

Heisler J.A., Weltzin J.F. (2006) Variability matters: towards a perspective on the influence of precipitation on terrestrial ecosystems. *New Phytologist* **172**: 189-192.

Jackson G.E., Irvine J., Grace J. (1999) Xylem acoustic emissions and water relations of *Calluna vulgaris* L. at two climatological regions of Britain. *Plant Ecology* **140**: 3-14.

Jentsch A., Kreylling J., Boettcher-Treschow J., Beierkuhnlein C. (2008) Beyond gradual warming: extreme weather events alter flower phenology of European grassland and heath species. *Global Change Biology* **14**: 1-13.

Jonasson S. (1988) Evaluation of the Point Intercept Method for the Estimation of Plant Biomass. *Oikos* **52**: 101-106.

Jonasson S., Chapin F.S. (1985) significance of sequential leaf development for nutrient balance of the cotton sedge, *Eriophorum vaginatum*. *Oecologia* **67**: 511-518.

Knapp K.A., Fay P.A., Blair J.M., Collins S.L., Smidth M.D., Carlistre J.D., Harper C.W., Danner B.T., Lett M.S., McCarron J.K. (2002) Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. *Science* **298**: 2202-2205.

Llorens L., Penuelas J., Beier C., Emmett B.A., Estiarte M., Tietema A. (2004) Effects of an experimental increase of temperature and drought in the photosynthetic performance of two ericaceous shrub species along a north-south European gradient. *Ecosystems* **7**: 613-624.

Luo Y., Gerten D., Maire G.L., Parton W.J., Weng E., Zhou X., Keough C., Beier C., Ciais P., Cramer W., Dukes J.S., Emmett B., Hanson P.J., Knapp A., Linder S., Nepstad D., Rustad L. (2008) Modeled interactive effects of precipitation, temperature and [CO₂] on ecosystem carbon and water dynamics in different climatic zones. *Global Change Biology* **14**: 1986-1999.

Margoils H., Oren R., Whitehead D., Kaufman M. (1995) Leaf area dynamics of conifer forests. In: Smith W.K., Hinckley T.M. (eds.) *Ecophysiology of coniferous forests*. Academic Press. San Diego, London. Pp 181-223.

Mänd P., Kull O., Peñuelas J., Hallik L., Nilson T., Duce P., Emmett B.A., Beier C., Estiarte M., Garadnai J., Kalapos T., Schmidt I.K., Láng E., Prieto P., Scarascia-Mugnozza G., Tietema A., Westerveld J.W. (2009) Responses of optical reflectance indices to experimental warming and drought in European shrublands along a North-South climatic gradient. *Remote sensing of the environment*. *In press*.

Mencuccini M., Grace J. (1995) Climate influence the leaf area-sapwood area relationship in Scots pine (*Pinus sylvestris* L.). *Tree Physiology* **15**: 1-10

Menzel A., Sparks T.H., Estrella N., Koch E., Aasa A., Ahas R., Alm-Kübler K., Bissolli P., Braslavska O., Briede A., Chielewski F.M., Crepensek Z., Curnel Y., Dahl Å., Defila C., Donnelly A., Filella Y., Jatzczak K., Måge F., Mestre A., Nordli Ø., Penuelas J., Pirinen P., Remosova V., Scheffinger H., Striz M., Susnik A., van Vliet A.J.H., Wielgolaski F.-E., Zach S., Züst A. (2006) European phenological response to climate change matches the warming pattern. *Global Change Biology* **12**: 1969-1976.

Michelsen A., Graglia E., Schmidt I.K., Jonasson S., Sleep D., Quarmby C. (1999) Differential responses of grass and dwarf shrub to long-term changes in soil microbial biomass C, N and P following factorial NPK fertilizer, fungicide and labile carbon to a heath. *New Phytologist* **143**: 523-538.

Mikkelsen T.N., Beier C., Jonasson S., Holmstrup M., Schmidt I.K., Ambus P., Pilegaard K., Michelsen A., Albert K.R., Andresen L.C., Arndal M.F., Bruun N., Christensen S., Danbæk S., Gundersen P., Jørgensen P., Kongstad J., Maraldo K., Primé A., Riis-Nielsen T., Ro-Poulsen H., Stevnbak K., Selsted M.B., Sørensen P., Larsen K.S., Carter M.S., Martinussen T., Miglietta F., Sverdrup H. (2008) Experimental design of multifactor climate change experiments with elevated CO₂, warming and drought – the CLIMAITE project. *Functional Ecology* **22**: 185-195.

Piao S., Friedlingstein P., Ciais P., Viovy N., Demarty J. (2007) Growing season extension and its impact on terrestrial carbon cycle in the Northern Hemisphere over the past 2 decades. *Global Biogeochemical Cycles* **21**: GB3018.
Doi: 10.1029/2006GB002888.

Penuelas J., Filella I. (2001) Responses to a warming world. *Science* **294**: 793-795.

Peñuelas J., Gordon C., Llorens L., Nielsen T., Tietema A., Beier C., Bruna P., Emmett B., Estiarte M., Gorissen A. (2004) Nonintrusive field experiments show different plant responses to warming and drought among sites, seasons, and species in a North–South European gradient. *Ecosystems* **7**: 598-621

Peñuelas J., Prieto P., Beier C., Cesaraccio C., De Angelis P., de Dato G., Emmett B.A., Estiarte M., Gorissen T., Garadnai J., Láng E.K., Kröel-Dulay G., Llorens L., Pellizzaro P., Riis-Nielsen T., Schmidt I.K., Sirca C., Sowerby A., Spano D., Tietema A. (2007) Response of plant species richness and primary productivity in shrublands along a north-south gradient in Europe to seven years of experimental warming and drought: reductions in primary productivity in the heat and drought year of 2003. *Global Change Biology* **13**: 2563-2581.

Prieto P., Penuelas J., Niinemets U, Ogaya R, Schmidt IK, Beier C, Tietema A, Sowerby A, Emmett B, Lang E K, Kroel-Dulay G, Lhotsky B, Cesaraccio C, Pellizzaro G, de Dato G, Sirca C, Estiarte M. (2009). Changes in the onset of shrubland species spring growth in response to an experimental warming along a north-south gradient in Europe. *Global Ecology and Biogeography*. *Accepted*.

Rustad L.E. (2008) The response of terrestrial ecosystems to global climate change: Towards an integrated approach. *Science of the total environment* **404**: 222-235.

Scurfield G. (1954) Biological Flora of the British Isles, *Deschampsia flexuosa* (L.) Trin. *Journal of Ecology* **42**: 225–233.

Watson A., Miller G.R., Green F.W.H.. (1966) Winter browning of Heather (*Calluna vulgaris*) and other moor plants. *Transactions of the Botanical Society of Edinburgh* **40**: 195-203.

Wan S., Hui., Wallace., Lou Y. (2005) Direct and indirect effects of experimental warming on ecosystem carbon processes in a tall grass prairie. *Global Biogeochemical Cycles* **19**: GB2014. Doi: 10.1029/2004GB002315.

White M.A., Running S.W., Thornton P.E. (1999) The impact of growing-season length variability on carbon assimilation and evapotranspiration over 88 years in the eastern US deciduous forest. *International Journal of Biometeorology* **42**: 139–145.

Figure 1: Climate at the temperate heath. Shown are daily averages of soil water content in 0-20 cm and daily accumulated precipitation (a), daily mean air temperature (b), and daily mean photosynthetically active radiation (c).

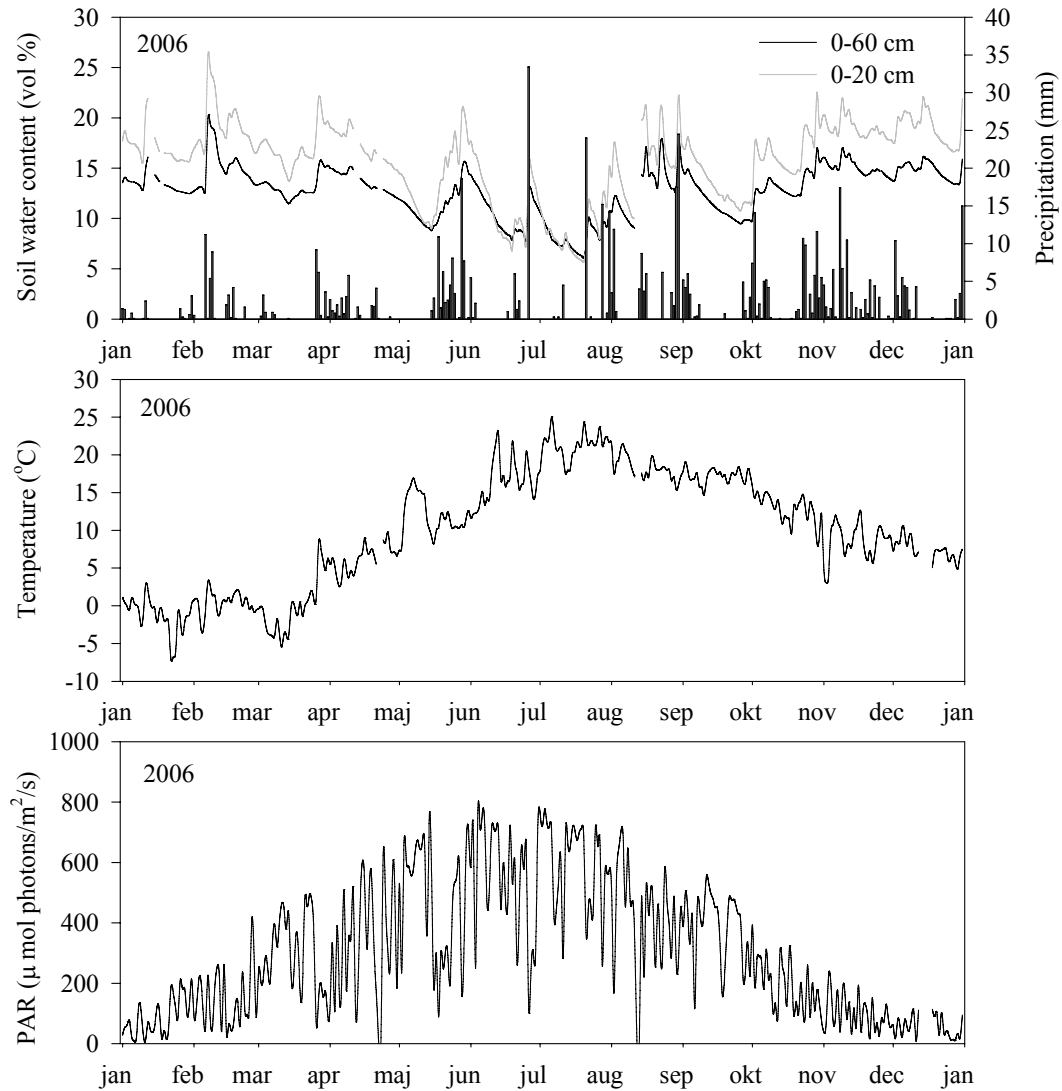


Figure 2: Photosynthetic and leaf characteristics. Panels left are *Calluna* and right are *Deschampsia*. Shown are means \pm standard error and the statistical difference between months are indicated with different letters ($p < 0.050$). Parameters are light saturated net photosynthesis (P_n), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), natural abundance of ^{13}C ($\delta^{13}C$) and soil water content in 0-60 cm (SWC).

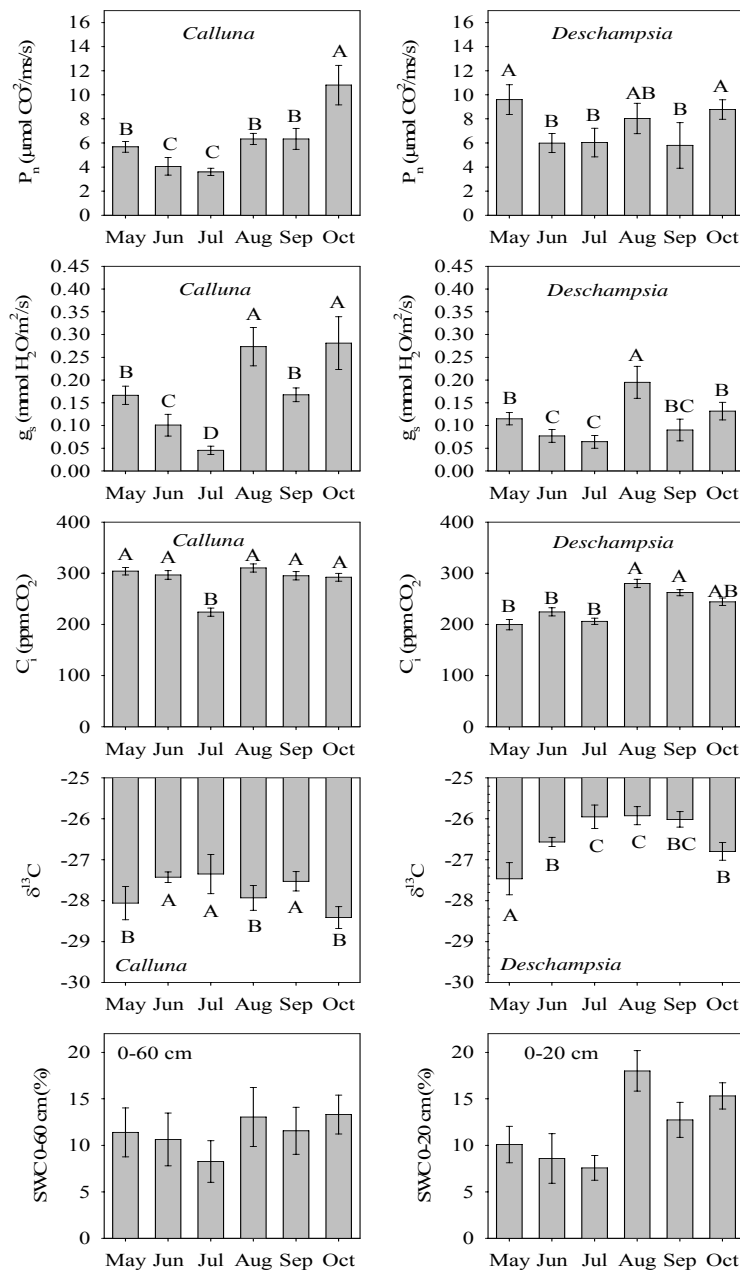


Figure 3: Leaf content of carbon and nitrogen and specific leaf weight. Panels left are *Calluna* and right are *Deschampsia*. Shown are means \pm standard error and the statistical difference between months are indicated with different letters ($p < 0.05$).

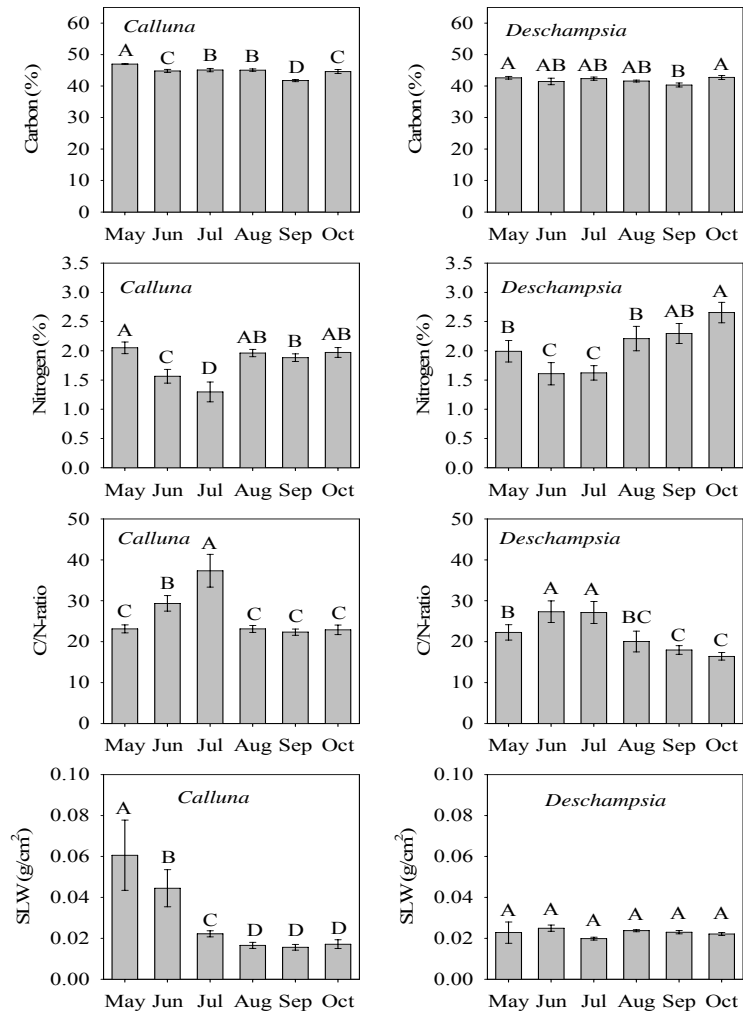


Figure 4: Photosynthetic capacity. Upper panels are *Calluna* and lower are *Deschampsia*. Shown are means \pm standard error and the statistical difference between months are indicated with different letters ($p < 0.050$). Parameters are the calculated maximum rate of Rubisco carboxylation (V_{cmax}), maximal rate of RubP regeneration (J_{max}), and rate of non-photorespiratory respiration (R_d).

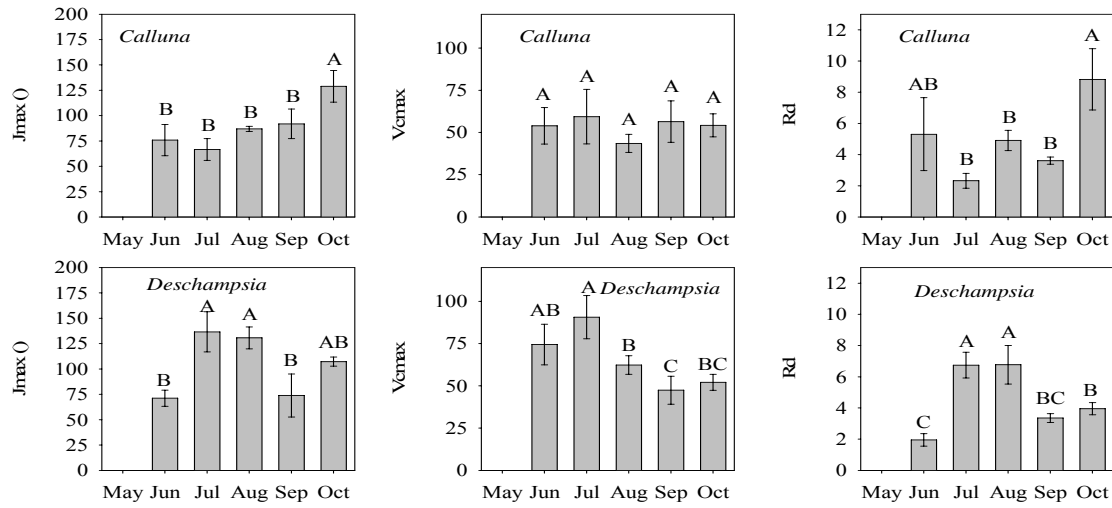
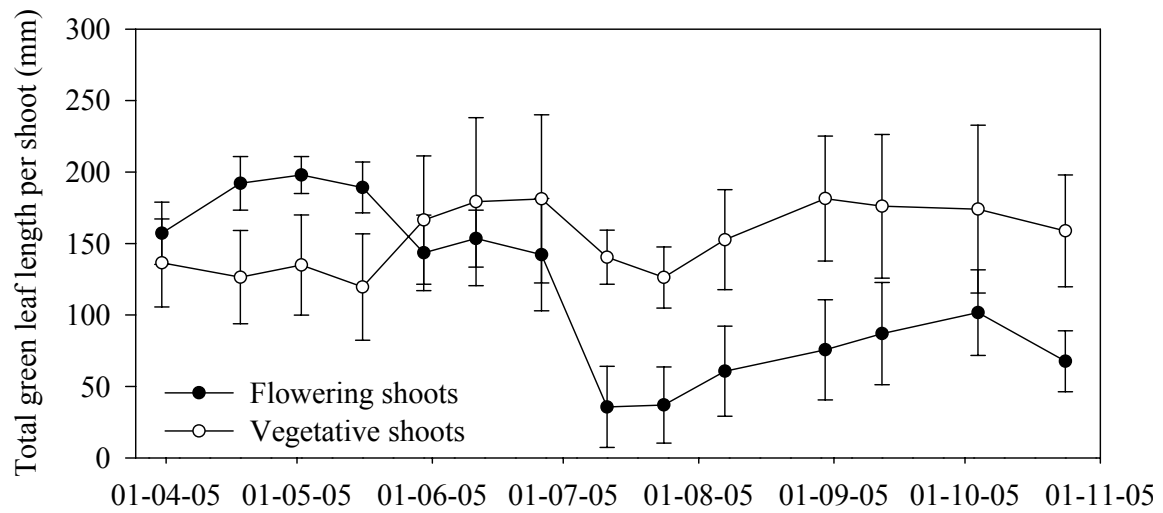


Figure 5: *Deschampsia* total green leaf length per shoot. Shown are mean \pm standard deviations. Open symbols are the vegetative shoots and closed the flowering shoots.



Paper II

Impact of elevated CO₂, warming and drought on photosynthesis of *Deschampsia flexuosa* in a temperate heath ecosystem.

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Manuscript

Impact of elevated CO₂, warming and drought on photosynthesis of *Deschampsia flexuosa* in a temperate heath ecosystem.

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List of abbreviations:

Carbon isotope ratio ($\delta^{13}\text{C}$), Experimental drought (D), Experimental elevated CO₂ (CO₂), Intercellular CO₂ concentration (C_i), Leaf carbon concentration (C), Leaf nitrogen concentration (N), Light saturated net photosynthesis (P_n), Passive night time warming (T), Soil water content (SWC), Stomatal conductance (g_s), Transpiration (T_r), Water use efficiency (WUE=P_n/T_r).

Keywords:

CLIMATE, multi factor experiment, net photosynthesis, heath ecosystem, *Deschampsia flexuosa*, FACE, drought, warming, grassland

Abstract

Various components of climate changes affect plant growing conditions at the same time. In order to predict the direction of responses to climate change and understand the underlying mechanisms it is important to combine the different environmental changes and investigate the interactions between factors. We investigated the photosynthetic response to elevated CO₂ (FACE), drought (water excluding curtains) and night time warming (IR-reflective curtains) in a multi-factorial experiment CLIMAITE in a temperate heath / grassland ecosystem. During the monthly campaigns the leaf gas exchange of CO₂ and H₂O were characterized with A/Ci curve, and leaf carbon and nitrogen concentration, $\delta^{13}\text{C}$, area and weight were determined. Elevated CO₂ increased net photosynthesis and water use efficiency in most months. Surprisingly elevated CO₂ did not decrease stomatal conductance. Warming alone had only small impact on net photosynthesis, but interacted with elevated CO₂, and this advanced the growing season in spring and increased photosynthetic carbon uptake in this period.

Changes in water availability strongly modulated the magnitude of photosynthesis in response to elevated CO₂. There was in general a close coupling between decreased soil water content and reduced stomatal conductance. Stomatal conductance increased more in plots exposed to elevated CO₂ than in ambient plots in periods with ample soil water content and this stimulated the plant carbon sequestration. Although water shortage progressively increased until severe drought was reached photosynthesis was sustained in elevated CO₂. However this was due to increased intercellular CO₂ concentration and not via stomatal regulations, which could have allowed for reduced water consumption and soil water savings. Aspects of plant carbon sink strength in a future climate scenario are discussed. Our result suggests elevated CO₂ to exert a strong control of available soil water which modulates plant carbon uptake. The carbon sinks in elevated CO₂ changes interactively with the advancement of growing season influenced by warming.

Introduction

Environmental changes caused by increased emissions of greenhouse gasses have been predicted to influence the stability of ecosystems worldwide (IPCC 2001). For terrestrial plants and ecosystems increases in atmospheric CO₂ concentration and air

temperature, and changes in precipitation patterns are expected to have strong impacts on carbon balance. Regional climate modelling show enhanced temperature and a decrease in precipitation in Denmark during summer resulting in longer drought periods and occasional incidences of high amounts of precipitation (Christensen and Christensen 2003). Effects of changes in the environment such as elevated CO₂, temperature and precipitation on plants and ecosystems have primarily been investigated as effects of a single factor or two factors in combination (e.g. Beier *et al.* 2004, for overview see Rustad 2008). Since all environmental changes will occur concurrently and all factors could interact it is important to study the effects of the factors alone and in combination in order to predict the outcome of multiple changes for plants and ecosystems processes in a changed climate.

Warming generally increases NPP (Dukes *et al.* 2005, Penuelas *et al.* 2007, Rustad *et al.*, 2001, Shaw *et al.*, 2002) with the strongest responses on sites with low aridity (Penuelas *et al.* 2007). Warming also increases the length of growing season (Cleeland *et al.* 2006, Menzel and Fabian 1999, Walter *et al.* 2002) and changes plant phenology (Harte and Shaw 1995, Menzel *et al.* 2006, Wan *et al.* 2005), increases soil nitrogen mineralization and availability (Rustad *et al.* 2001), reduces soil water content (Morgan *et al.* 2004, Volk *et al.* 2000, Wan *et al.* 2005) and affects species composition and community structure (Shaver *et al.* 2000, Wan *et al.*, 2005). The indirect effects on photosynthesis and productivity have been argued to be more important than direct effects (Körner 2000, Lou 2007, Morgan *et al.*, 2004, Shaver *et al.* 2000).

Elevated CO₂ predominantly decreases stomatal conductance, stimulates the light saturated net photosynthesis and increases plant water use efficiency (Ainsworth and Rogers 2007, Ainsworth and Long 2005, Curtis 1996, Curtis and Wang 1998). At elevated CO₂, the excess capacity for carboxylation is reduced through reduction of Rubisco content, or nitrogen redistribution of the excess N invested in Rubisco (Drake *et al.* 1997, Parry *et al.* 2003). This response leads to the decrease in leaf nitrogen content and increased leaf C/N ratio in many studies, as summarized by e.g. Ainsworth and Long (2005) or Ainsworth and Rogers (2007). The magnitude of the response of photosynthesis to elevated CO₂ is modulated by nitrogen supply and by constraints of the capacity to utilize the photosynthates (Ainsworth and Rogers 2007). The importance of the plant

water relations via the CO₂ water savings has been stressed in particular in grasslands and forest exposed to elevated CO₂ (Körner 2000, Leuzinger and Körner 2008, Morgan *et al.*, 2004, Volk *et al.* 2000).

In response to mild to moderate drought decreased stomatal conductance to save transpirational water loss is seen, while loss of ribulose biphosphate (RuBP) and thus decreased the net photosynthesis dominates at severe drought (Ciais *et al.* 2005, Flexas and Medrano 2002, Penuelas *et al.* 2007). However, elevated CO₂ improves the water use efficiency via decreased stomatal conductance (Medlyn *et al.* 2001, Morison and Gifford 1984). This leads to less soil moisture depletion in elevated CO₂ (Drake *et al.* 1997, Wall *et al.* 2001) and in habitats with periodical drought enhanced CO₂ could extend the period of active growth. Soils under grassland exposed to elevated CO₂ maintain higher moisture (Fredeen *et al.* 1997, Niklaus *et al.* 1998) and water status may strongly co-determine plant biomass responses to CO₂ enrichment (Acock and Allen 1985, Field *et al.* 1997, Gifford 1992, Koch and Mooney 1996).

The study is a part of the CLIMAITE experiment (Mikkelsen *et al.* 2008) in which we manipulate temperature, soil moisture and CO₂ by passive night time warming (T), periods of drought (D) and elevated CO₂ (CO₂) during the day, alone and in all combinations. To investigate the responses of photosynthesis of the dominant grass *D. flexuosa* we performed monthly campaigns of leaf gas-exchange, and analyzed leaf characteristics of carbon, nitrogen and $\delta^{13}\text{C}$. These parameters allowed for analysis of the accumulated responses of carbon uptake, water status, and nitrogen balance in the experiment. We evaluated the impact of the three environmental factors alone and in combination, in order to investigate possible shifts in importance of the treatments during the growing season.

We hypothesized that:

- Warming (T) would advance the spring and also extend the growing season in the autumn
- Warming would increase carbon uptake and increase the water consumption
- Summer drought (D) would decrease photosynthesis and stomatal conductance

- Elevated CO₂ (CO₂) would increase photosynthesis and decrease stomatal conductance
- Responses to the climatic factors (T), (D) and (CO₂) would be additive, and hence, that there would be no interaction between climatic factors regarding their effects on plant performance.

Materials and Methods

Site and experimental setup

The experimental site is a dry heathland ecosystem in North Sealand, DK, dominated by the C3 grass *Deschampsia flexuosa* and the evergreen dwarf shrub *Calluna vulgaris*. The experimental treatments were elevated CO₂ (CO₂), passive nighttime warming (T), summer drought (D), all combinations, (TD), (TCO₂), (DCO₂), (TDCO₂) and un-manipulated control (A), replicated in six blocks in a split-plot design. Each block consisted of two octagons of 8m diameter, each divided in four plots. In one octagon CO₂ was elevated to 510 ppm with the FACE (Free Air CO₂ Enrichment, Miglietta *et al.*, 2001) technique and in the other octagon the CO₂ level was ambient. In each octagon automatized infrared reflective curtains covered one half during night, preserving a proportion of the daily incoming radiation energy, which increased the night air temperature by up to 4°C, on average 1-2 °C. Perpendicular to the IR-curtains was a rain excluding curtain which automatically was activated by rain during the selected drought periods. In each experimental plot the soil temperature was measured continuously in 2 and 10 cm depth and the soil moisture in 0-20 and 0-60 cm depth. Two climatic stations recorded precipitation, air temperature, photosynthetically active radiation and speed and wind directions (Mikkelsen *et al.* 2008). The CO₂ and warming treatments were started 3rd October 2005. The drought was started 3rd July 2006 and was continued for 5 weeks until 4th of August when soil water content reached c. 5 % water content in the top 20 cm of the soil. For further description of site and experimental setup see Mikkelsen *et al.* (2008).

Leaf gas exchange

Gas exchange measurements of CO₂ and H₂O fluxes were conducted *in situ* by using two LI-6400's (LI-COR Biosciences, Lincoln, Nebraska, USA) connected to standard 2x3 cm chambers with LED light source (6400-02B) and were carried out in 2006: May 11-18th, June 12-16th, July 10-13th, August 14-21st, September 11-22nd and October 9-20th. Only fully expanded, healthy leaves from the top of the vegetation were selected. Pilot studies resulted in the following methodology securing highly reproducible measurements. About 10-20 *D. flexuosa* leaves were positioned side by side and gently fixed by hair needles during each of the monthly periods of measurements. Leaf cuvettes were then fixed to the samples using flexible arms (Magic Arm 143, Manfrotto, Italy). During the following harvest care was taken to ensure that area and weight were determined on exactly the same material as inserted in the leaf cuvette.

The protocol for CO₂ response curves were optimized and tested. Samples were acclimated 2-4 minutes at ambient CO₂ (380 ppm), until net photosynthesis and stomatal conductance were stabilized (± 1 CV over 30 sec). The CO₂ response curves were measured by stepping CO₂ down (from 380 ppm to 300, 200, 100, 50 ppm and then back to 380 ppm for 4 of minutes re-acclimation, until initial state was reached again. Then CO₂ was stepped up to saturation (from 380 ppm to 450, 510, 650, 800, 1000, 1200, 1400 ppm) at the saturating light level of 1500 $\mu\text{mol photons/m}^2/\text{s}$. The LI-COR Auto program 'A/Ci-curve' was used (Settings: Min 45 and max 60 sec, reference CO₂ stable in 10 sec with CV<1%, C_i stable in 10 sec with CV<1%, IRGA matching performed at each step). Block temperature was held constant at 25°C and sample relative humidity was stabilized at 45-55% during measurements. All measurements were area corrected. Gas-exchange parameters as the light saturated net photosynthesis (P_n), transpiration (T_r), Water use efficiency (WUE=P_n/T_r), stomatal conductance (g_s), intercellular CO₂ concentration (C_i), were extracted from the CO₂ response curves at reference CO₂ level, CO₂Ref = 380 ppm CO₂ in non-FACE plots and at CO₂Ref = 510 ppm CO₂ in FACE plots.

Leaf weight, area, nitrogen, carbon and $\delta^{13}\text{C}$

Immediately after harvest digital pictures were taken of the leaves flattened by transparent acrylic glass beside a brick of known area. The photographs were converted to black and white and loaded into a pixel counting program (Bitmap, S. Danbæk,

Department of Biology, University of Copenhagen) and the leaf areas were determined. Then the fresh weight was determined. The dry weight was determined after oven drying at 80°C for 48 hours. The plant material was analyzed for C and N concentration and ^{13}C natural abundance ($\delta^{13}\text{C}$) with a Eurovector CN analyzer coupled to an Isoprime isotope ratio mass spectrometer. During analysis, the internal reference gas was calibrated against certified standards from the International Atomic Energy Agency, and plant material calibrated against certified standards was used as working standard. Leaf $\delta^{13}\text{C}$ is expected to increase in plant with higher WUE. From dry weights and leaf area the specific leaf area, SLA, were calculated. Leaf water content was calculated from fresh and dry weights.

Statistics

Analyses of variance were made in SAS using the proc mixed to test for effects of the fixed factors: Month, (T), (D), (CO_2) and their interactions (T*D), (T* CO_2), (D* CO_2), (T*D* CO_2). To take into account the split-plot design, the plot was nested within octagon and octagon nested within block and these two terms set as random factors. Pre-treatment data were initially included in the full model as co-variates and handled as a fixed factor. The model was reduced by progressively dropping in-significant fixed factors, starting with the higher order interactions until only significant ($P < 0.05$) or near significant terms ($p < 0.10$) remained. Significance levels are reported as a tendency with † when $p < 0.10$ and as significant with * when $p < 0.05$, ** when $p < 0.001$ and *** when $p < 0.0001$. All data were normal distributed and some parameters were transformed to fulfill the assumption of variance homogeneity. To interpret the direction of the significant responses we compared the least squared means.

Results

Environmental conditions

Snow melted in late March and from early April the mean daily temperature in 2m height gradually increased from 0°C to 25°C in late July. 2006 was the warmest year ever recorded in Denmark, 9.4°C (www.DMI.dk) and mean daily air temperatures were above

11°C in late November (Fig. 1). Warming treatment increased the 24h mean daily nighttime versus daytime warming ?? temperature 1-2°C and there was 33% higher number of accumulated growing degree days, GDD, from 1st April to 15th May, and an annual 7% increase in GDD warmed plots compared to controls. The warming treatment increased the maximum temperatures in the late night/early morning in both the air and the soil, and the effects of warming treatment gradually reduced during the day. In the air, the effect of the warming treatment disappeared 3–5 h after sunrise and rapidly built up again after sunset, while in the soil the warming effect were less dynamic and some warming is sustained throughout the day. (Mikkelsen *et al.* 2008). The CO₂ concentrations in the FACE plots were elevated to 510 ppm during daytime, but not during night with monthly average concentrations at 500-520 ppm CO₂ (Mikkelsen *et al.* 2008). Despite some precipitation, the soil water content (SWC) decreased from 17.2 % in April to 8.8 % July in controls. This natural low SWC was extended with the experimental drought period (D) in which SWC decreased even further towards August. In this period the non-drought plots benefitted from some incidents of precipitations. Rewetting increased the SWC to 18% in August, 13.6 % in September to 16.5% in October in the controls. Soil water content in 0-20 cm depth showed decreased values in warming treatments in May, June, August and September and in response to experimental drought in July (Fig. 2). There was a significant interaction effects D*CO₂ in July revealing increased SWC values in response to drought only when combined with elevated CO₂ (Fig. 2).

Seasonal changes in leaf gas-exchange

Net photosynthesis generally showed high levels across treatments in May (range 8-16 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$), lower levels during June, July, August, and September (range 4-13 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$) and again high levels in October, (range from 6 to 11.5 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$), (Fig. 3). Stomatal conductance levels in the un-manipulated control plots decreased during May, June and to July from 0.116, 0.077 to 0.066 $\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$. In August the g_s in the controls was again high 0.195 $\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$, it dropped in September 0.090 $\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$, and was intermediate in October, 0.132 $\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$ (Figure 4). The water

use efficiency, WUE, across season varied from about 3 to 9 $\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$, with a seasonal low in August and highest values in May and October (Figure 5).

Besides a clear effect of month for all parameters (all $p < 0.0001$), the elevated CO_2 significantly increased P_n , g_s , and WUE (all $p < 0.0001$) across season.

Treatment responses in leaf gas exchange

In May P_n were higher in treatments with (T) and (CO_2) combined as compared to (T) or (CO_2) alone, leading to a significant interaction ($T \times \text{CO}_2$) (Fig. 3). There were no effects on g_s , but the WUE increased by elevated CO_2 ($p < 0.0001$) and by warming ($p < 0.031$) (Fig. 4 and 5). In June, P_n and g_s increased in elevated CO_2 ($p < 0.0061$, Fig. 3; $p < 0.0031$, Fig. 4). The significant ($T \times \text{CO}_2$) interaction ($p < 0.0017$, Fig. 3; $p < 0.0552$, Fig. 4) for P_n and g_s was due to decreases in T and TD as compared to control, but increases when combined with elevated CO_2 in T CO_2 and D CO_2 (Fig. 3 and Fig. 4). There was no effect on WUE (Fig. 5). In July the P_n increased in elevated CO_2 ($p < 0.0277$, Fig. 3), but decreased in response to drought ($p < 0.0397$). The g_s was also decreased by drought ($p < 0.0043$, Fig. 4). The WUE increased in elevated CO_2 ($p < 0.0254$, Fig. 5). In August the P_n was decreased in response to drought ($p < 0.0415$, Fig. 3). There was an interaction between warming and elevated CO_2 ($T \times \text{CO}_2$) on P_n ($p < 0.0233$, Fig. 3) and on WUE ($p < 0.0321$, Fig. 5), which revealed that responses to warming were bi-directional as warming increased P_n and WUE, but only in combination with elevated CO_2 in the T CO_2 and TDCO $_2$ treatments. In September, the drought increased P_n and g_s ($p < 0.0030$, Fig. 3; $p < 0.0413$, Fig. 4). Elevated CO_2 increased P_n ($p < 0.0427$, Fig. 3) and WUE ($p < 0.0192$, Fig. 5). In October, elevated CO_2 increased the P_n , g_s and WUE ($p < 0.0001$, Fig. 3; $p < 0.0389$, Fig. 4; $p < 0.0001$, Fig. 5).

Deschampsia leaf characteristics

The leaf characteristics of *Deschampsia* were related to the bi-phasic growth pattern, with two peaks of green biomass before and after flowering. The first phase was characterized by leaf length extension in May and June and a large dieback in July. The leaf carbon was stable at 40-42 %, whereas nitrogen decreased from 1.69 to 1.44 % leading to an increasing C/N-ratio from 26.3 to 30.3 (Fig. 6). Leaf area per DW expanded as SLW

increased from 38.6 to 50.3 cm²/g. The leaf water content was 36-40 %. The second phase of were produced in August and growth of these continued during September and October. There was a balance between leaf carbon (40-42 %) and nitrogen (2.27-2.46 %) which led to a stable C/N-ratio at 17.9-18.9. The leaf area expanded as SLW increased from 41.5 to 44.1 cm²/g. The leaf water content was between 27-30%.

In relation to treatments, the carbon concentration changes were relatively small, but C decreased or tended to decrease in response to elevated CO₂ in July, September and across season ($p < 0.0768$, $p < 0.049$, $p < 0.0764$, data not shown). Moreover there was a significant interaction between drought and elevated CO₂ (D*CO₂) in August ($p < 0.035$, data not shown) due to a decrease in leaf carbon only when drought and elevated CO₂ were combined.

Elevated CO₂ decreased the nitrogen concentration in May, June, July and September ($p < 0.0117$, $p < 0.065$, $p < 0.0136$, $p < 0.0053$, data not shown). Drought increased or tended to increase leaf nitrogen concentration in September and October ($p < 0.0006$, $p < 0.0588$, data not shown) and a tended towards significant interaction D*CO₂ in October also revealed an increase in drought, but only in ambient CO₂ plots ($p < 0.0657$, data not shown).

The leaf C/N ratio increased in elevated CO₂ in May, June, July and September ($p < 0.0175$, $p < 0.0336$, $p < 0.0292$, $p < 0.0037$, Fig. 6). Drought decreased the C/N ratio in September ($p < 0.0005$, Fig. 8) and the interaction D*CO₂ in October ($p < 0.045$) revealed an increase C/N ratio in elevated CO₂, but not when combined with drought. In general the changes in the C/N ratios were governed by differences in nitrogen concentrations.

The leaf water content were increased in elevated CO₂ in May, June, and September ($p < 0.0055$ $p < 0.0092$, $p < 0.0018$, data not shown) and decreased in drought in July, September and October ($p < 0.0425$, $p < 0.0001$, $p < 0.0094$, data not shown). There were no significant effects on SLW.

The leaf $\delta^{13}\text{C}$ was in all months decreased in elevated CO₂ due to the lower $\delta^{13}\text{C}$ signature of the industrial CO₂ gas (c. -28.1 $\delta^{13}\text{C}$ as opposed to -8.0 in ambient air) added to the FACE plots ($p < 0.0001$, Fig. 7). Warming decreased the $\delta^{13}\text{C}$ in August, September and October ($p < 0.0100$, $p < 0.0077$, $p < 0.0202$, Fig. 7) and the interaction T*CO₂ were significant in May, June and August ($p < 0.0052$, $p < 0.0029$, $p < 0.0234$, Fig. 7) due to a

decrease in $\delta^{13}\text{C}$ only when warming and elevated CO_2 were combined. The interaction $\text{D} \times \text{CO}_2$ were significant in July ($p < 0.0404$, Fig. 7) due to an increase in $\delta^{13}\text{C}$ in response to drought only when combined with elevated CO_2 .

Discussion

Effects of elevated CO_2 on photosynthetic parameters

The net photosynthesis increased in elevated CO_2 mainly via increased intercellular CO_2 concentrations during most of the growing season. When water availability was favorable, as in June and October, g_s and P_n increased in elevated CO_2 . The increased photosynthesis response was concurrent with an increased C/N ratio, indicating that some of the surplus assimilated carbon was allocated to leaf growth without corresponding incorporation of nitrogen. This pattern was evident in the first growth phase (May to July) and most of the second (August to October), except for the very beginning of the latter phase where the new leaves were developing. Also the increased WUE were primarily caused by the increased photosynthesis in elevated CO_2 . Hence, it seems that after one year of treatment, the elevated CO_2 were beneficial for the primary productivity of *Deschampsia* in this dry heathland ecosystem, in line with most findings on photosynthetic responses to elevated CO_2 (Ainsworth and Rogers 2007, Ainsworth and Long 2005). However, contrary to expectations the stomatal conductance increased periodically in elevated CO_2 showing the plasticity in this parameter.

Improved water relations in elevated CO_2 were discussed in e.g. Körner (2000), Morgan *et al.* (2004) and Volk *et al.* (2000). Volk *et al.* (2000) suggested that, depending on the degree of drought and the species-specific water demand, the net response of stomatal conductance could be either positive, negative or zero, owing to the antagonistic nature of direct (reducing) and indirect (enhancing) CO_2 effect on g_s . Either way elevated CO_2 increased the photosynthetic carbon uptake.

In September and October elevated CO_2 increased P_n led to increased C/N-ratio, but not via changes in g_s , leading to significantly increased WUE. This is in contrast to June, where SWC also were favorable, but here the g_s increased in elevated CO_2 , allowing for a higher P_n despite the higher water loss. This is in line with the favorable water relations and early season start, as observed in TCO₂ plots in May and June, which

allowed for higher photosynthetic CO₂ uptake. This pattern provides further support for the notion that limitations on photosynthetic improvement in elevated CO₂ are governed by water relations, as pointed out in several other FACE experiments (Körner 2000, Leuzinger and Körner, Morgan *et al.* 2004, Volk *et al.* 2000). However, in contrast to several of these studies the stimulation of photosynthesis via stomatal conductance regulations in elevated CO₂ were not seen during the severe drought.

The reason for that is probably that plants in elevated CO₂ have depleted the available soil water resources during the earlier luxurious growth, but also that the first phase leaves are in the process of wilting down. When water again became available, the new formed leaves increased their photosynthesis vigorously then in the TCO₂ treatments.

Effects of warming on the photosynthetic parameters

The passive nighttime warming treatment showed a diurnal pattern and some air temperature increase during early daylight hours. To the degree this translated into increased leaf temperature this likely caused some short time duration of direct effects on leaf photosynthetic metabolism. Although the adopted measurement protocol were not suitable to detect such effects then it may be possible that the increased temperature have increased the photosynthesis rate (Sage and Kubien 2007) and leaf respiration (Atkin and Tjoelker 2003) during this short period of direct warming.

The warming treatment caused an earlier start of the growing season via indirect effect, in line with other studies (Cleeland *et al.* 2006, Menzel and Fabian 1999, Walter *et al.* 2002). In May the net photosynthesis of *Deschampsia* was high and further stimulated by warming when combined with elevated CO₂, which caused the significant T*CO₂ interaction. This accumulated and decreased the $\delta^{13}\text{C}$. Compared to May the overall level of net photosynthesis in June was much lower concurrently with SWC, but net photosynthesis was still higher when warming and elevated CO₂ was combined. Beside the phenological effects in May and June the photosynthetic response was also affected by the lower water availability in June. This caused the concurrently higher net photosynthesis when warming and elevated CO₂ was combined. This is further supported by lack of TCO₂ effects on WUE and the increases in g_s in warming and elevated CO₂,

and decreases in $\delta^{13}\text{C}$ in TCO₂ plots. This clearly indicates the positive impact of CO₂ on plant water relations in a period when the SCW progressively decreased both due to environmental conditions and the additional water consumption governed by the prolonged growing season. Interestingly, nocturnal warming have been demonstrated to increase photosynthesis in elevated CO₂ via increased night-time respiration which counteracted the down-regulation of photosynthesis by increasing the rate of utilization or export of photosynthates (Turnbull *et al.* 2002, Turnbull *et al.* 2004).

Warming also shifted the timing of the second phase leaves, which caused an enhanced P_n and a decreased $\delta^{13}\text{C}$ in most of the warmed plots in August, September and October. In this period water supply was sufficient while there were no effects of warming on photosynthesis or stomatal conductance per se. This indicates that the effect of warming on $\delta^{13}\text{C}$ was caused by other factors, e.g. an earlier leaf emergence and faster development.

Effects of drought on the photosynthetic parameters

The importance of water relations via CO₂ water savings has been stressed particular in grasslands (Morgan *et al.* 2004, Volk *et al.* 2000). It has been demonstrated that soils under grasslands exposed to elevated CO₂ maintain higher moisture (Fredeen *et al.* 1997, Niklaus *et al.* 1998) with potential to increase the period of active growth (Robredo *et al.* 2007).

In July the soil water content in 0-20 cm depth decreased to a seasonal low and this led to water shortage in *Deschampsia* across all treatments, with seasonal low values for g_s . Shortly after the experimental drought was started (1-2 weeks) the water limitation was intensified decreasing the SWC and further decreased the g_s and P_n in the experimental drought plots. During the measurement campaign in July the SWC were marginally higher when elevated CO₂ and experimental drought were combined indicating that some water saving had taken place. However previous high water consumption in elevated CO₂ and likely higher LAI resulted in lower water availability for the individual leaves in elevated CO₂ plots. Therefore the water limitation was particular pronounced when experimental drought was combined with elevated CO₂, as seen by the D*CO₂ interaction on leaf $\delta^{13}\text{C}$. This contrast other results from other

grasslands where elevated CO₂ resulted in soil water savings (Bremer *et al.* 1996, Morgan *et al.* 2004, Niklaus *et al.* 1998b) brought about by decreased stomatal conductance extending the period with sustained photosynthesis in response to elevated CO₂ (Ainsworth and Long 2005, Lauber and Körner 1997). Further, during July-August a large proportion of the *Deschampsia* first phase leaves died back and was replaced by second phase leaves in August, with lower leaf water content and higher N concentration than in the previous phase. However, in August the leaf abundance and cover was lower in drought plots (KA pers.obs).

In August, the measurements were conducted after the end of the experimental drought, and precipitation events had increased the SWC, allowing for the higher levels of g_s and P_n in August compared to July. However, the drought significantly decreased the g_s and P_n in August. Interestingly, this did not accumulate into a decreased C/N ratio or effect on $\delta^{13}C$ in August, indicating that the general pattern of leaf dieback in July may have been more pronounced in drought plots, leaving few or no leaves surviving. However, leaves in all treatments in August were most likely newly formed leaves with high nitrogen content and low C/N, and by this time the leaves were unaffected by treatment.

The interaction between drought and elevated CO₂ (D*CO₂) on $\delta^{13}C$ in July clearly indicates the impact of water stress accumulation in the drought period in droughted plots which also were exposed to elevated CO₂. The responsiveness of stomatal conductance was minor at this time as this parameter was at its seasonal low. Therefore the $\delta^{13}C$ change was mainly due to effects on C assimilation in July and earlier. Elevated CO₂ did to some degree mitigate the negative effect of drought on net photosynthesis which seems in conflict with the increasing water stress seen as decreased values of $\delta^{13}C$ in July. However, if elevated CO₂ increased aboveground biomass, as show elsewhere for grassland (Morgan *et al.* 2004) then the water consumption in elevated CO₂ may have increased faster than the water savings obtained through decreased stomatal conductance during the drought. This explains why *Deschampsia* did not benefit from the very marginal water savings in elevated CO₂ treatments.

In September re-wetting had occurred over a longer time period and the P_n increased in response to elevated CO₂, and also to less degree increase in drought plots.

The increase in the former experimental drought plots may be related to increases in g_s , clearly indicating better water accessibility. The decreased leaf area per ground area in the former drought plots during August to September allowed for more luxurious water consumption. This may be viewed as a short term transient stimulation in response to rewetting after drought, and it did not accumulate into measurable growth via decreased C/N ratio or changed $\delta^{13}\text{C}$ in drought plots in September.

Drought had effects which lasted for beyond the experimental drought period, particularly in plots also exposed elevated CO_2 as revealed by the significant interaction ($\text{D} \times \text{CO}_2$) in October. The decreased C/N ratio persisted in October in the treatments DCO_2 and TDCO_2 , clearly indicating that even though P_n was increased in response to elevated CO_2 this did not increase the C/N ratio in the late autumn, when elevated CO_2 were combined with experimental drought.

Seasonal effects of treatments and multifactor responses

Together, these findings demonstrate strong impact of the treatments, both as direct and indirect effects. Passive nighttime warming caused both an earlier onset of the growth both during the primary growth phases, in spring, and in late summer. Net photosynthesis was increased in elevated CO_2 in most months and in particular during May and June, but also in August, when warming and elevated CO_2 interacted. Soil water content progressively decreased in May through June, but elevated CO_2 sustained high P_n despite no or even increased stomatal conductance. This is surprising and indicates that elevated CO_2 did not alleviate drought via water savings by stomatal closure in same way as expected (Robredo *et al.* 2007, Volk *et al.* 2000). This could be due to allocation of assimilates to root growth in elevated CO_2 and in the combined treatment of elevated CO_2 and warming in order to sustain this high water consumption. The experimental drought had a negative effect on g_s and P_n , but the re-vitalization after rewetting reversed this. The unusual warm autumn temperatures likely postponed senescence in all treatments and particular high carbon assimilation were sustained in elevated CO_2 . However the opportunistic water consumption of *Deschampsia* in elevated CO_2 and the strategy of wilting down when SWC became unfavorable also support water savings not to be realized.

Even when the growth patterns are taking into account, then the impact of (T), (D) and (CO₂) differed in strength during season. The combination of treatments often caused unpredictable responses seen as the significant interactions. One directional effect was produced by the factors (CO₂) and (D) always increasing or decreasing net photosynthesis, whereas the factor (T) alone did not produce any significant effects on net photosynthesis. This pattern supports that (CO₂) and (D) are drivers that more directly impact the photosynthetic process in an additive way, whereas (T) does not. Hence, the effects of (T) were more indirect via changes in growth patterns and water balance. This response pattern led to the interaction effects of elevated CO₂ and warming (T*CO₂) and the factors acted in a synergistic way.

Warming promoted early season growth (May) and as SWC was favorable in early summer, warming extended the period of increased P_n (June) and increased the responsiveness to rewetting after the natural drought which increase the P_n (August). W

While no synergistic effects of elevated CO₂ and warming were seen in late season, there was a main effect of elevated CO₂ on net photosynthesis, probably due to the unusual warm autumn temperatures. Therefore we can not exclude that the strength of the warming treatment could be most pronounced when temperatures becomes unfavorable for productivity in ambient plots, but measurements even later than mid-October are needed to verify this.

The photosynthetic stimulation caused by enhance SWC in June was linked to precipitation events or ample soil water in general and not to water savings in elevated CO₂. This may be an initial response in the experiment if increased transpiration via warming of more dense canopies, and air humidity changes takes place. Contrary to our results other studies have clearly demonstrated water savings by grasses in elevated CO₂ (Bremer *et al.* 1996, Morgan *et al.* 2004, Niklaus *et al.* 1998b) and positive effects on carbon sequestration in grasslands (Ainsworth and Long 2005, Lauber and Körner 1997). We would then expect increased photosynthetic carbon uptake in this species in wet years. However, data from coming years differing in precipitation regimes are needed to verify this. Hence, photosynthetic and productivity responses to elevated CO₂ are influenced by species identity and resource availability (Körner 2006, Nowak *et al.* 2004).

Some of the assimilated carbon in elevated CO₂ was built into leaves increasing the C/N ratio in elevated CO₂. Only in August and October the C/N-ratio were stable, which suggests that nitrogen uptake matches the carbon input by photosynthesis in these periods. As some level of soil moisture is necessary for the mineralization processes this process likely influence the increasing C/N ratios in the increasingly dryer June and July. In the longer term nitrogen limitation very likely constrains the potential for increased productivity in response to CO₂ (Lou *et al.* 2004, Finzi *et al.* 2002, Hungate *et al.* 2006, Reich *et al.* 2006), but in this dry ecosystem it may difficult to separate this effect from the ample water conditions.

The heathland ecosystem are fully developed with leaf area index and fine root renewal at steady state, similar to mature forest systems as conceptualized by Körner (2006). In such system the stimulation in biomass production may to a large degree result from CO₂ induced improved water relations and to a less degree directly from elevated CO₂. The productivity patterns in the short term were stimulated in elevated CO₂ in periods with sufficient SWC. Further complexity is added by the biphasic growth pattern of the dominant grass which allowed for leaf die-back when SWC was reduced and by the shifts in growing season due to warming. Hence, this species is highly responsive to changes in amount and timing of precipitation. Repeated time series analysis in multi-factorial experiments over many years are needed to reveal longer term changes in productivity.

Conclusion

Elevated CO₂ and warming interacted to advance the growing season and increase the net photosynthesis. Progressive drought down to the wilting point decreased photosynthesis and stomatal conductance and caused pronounced dieback of green leaves. When drought was combined with elevated CO₂ no decrease in stomatal conductance was seen. Hence the improved water use efficiency was stimulated via increased photosynthesis. Net photosynthesis was stimulated by elevated CO₂ particularly in periods with sufficient water available, and also in response to rewetting. The unusual warm autumn with frequent incidents of precipitation stimulated net photosynthesis particularly in elevated

CO₂, and postponed senescence beyond October. Water relations clearly modulated the magnitude of the net photosynthesis in elevated CO₂. With time the concerted impact of the changes in elevated CO₂, drought and warming on the ecosystem processes and pools likely approach and a steady state. The plasticity in photosynthetic characteristics, growth pattern and opportunistic resource exploration the grass *Deschampsia* are clearly advantageous characteristics for plants exposed to climatic changes. However, in short term, the limitations by low soil water content and stimulation by warming clearly structures the response of photosynthesis in elevated CO₂ in this grassland species.

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References

Acock B., Allen L.H. (1985) Crop responses to elevated carbon dioxide concentrations. In: Strain BR, Cure JD (eds) Direct effects of increasing carbon dioxide on vegetation. Office of Energy Research, Department of Energy, Washington, pp 53–97.

Aerts R., Berendse F., de Caluwe H., Schmitz M. (1990) Competition in heathland along an experimental gradient of nutrient availability. *Oikos* **57**: 310-318.

Ainsworth E.A., Davey P.A., Hymus G.J., Osborne C.E., Rogers A., Blum H., Nosberger J., Long S.P. (2003) Is stimulation of leaf photosynthesis by elevated carbon dioxide concentration maintained in the long term? A test with *Lolium perenne* grown for 10 years at two nitrogen fertilization levels under Free Air CO₂ Enrichment (FACE). *Plant Cell & Environment* **26**: 705-714.

Ainsworth E.A., Long S. (2005) What have we learned from 15 years of Free-Air CO₂ Enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New Phytologist* **165**: 351-372.

Ainsworth E.A., Rogers A. (2007) The response of photosynthesis and stomatal conductance to rising [CO₂]: mechanisms and environmental interactions. *Plant, Cell & Environment* **30**: 258-270.

Atkin O.K., Tjoelker M.G. (2003) Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends in Plant Science* **8**: 343-351.

Beier C., Emmett B., Gundersen P., Tietema A., Penuelas J., Estiarte M., Gordon C., Gorissen A., Llorens L., Roda F., Williams D. (2004) Novel approaches to study climate change effects on terrestrial ecosystems in the field: Drought and passive nighttime warming. *Ecosystems* **7**: 583-597.

Bernacchi C.J., Morgan P.B., Ort D.R., Long S.P. (2005) The growth of soybean under free air [CO₂] enrichment (FACE) stimulates photosynthesis while decreasing in vivo rubisco capacity. *Planta* **220**: 145-154.

Bernacchi C.J., Leakey A.D.B., Heady L.E., Morgan P.B., Dohleman F.G., McGrath J.M., Gillespie K.M., Wittig V.E., Rogers A., Long S.P., Ort D.R. (2006) Hourly and seasonal variation in photosynthesis and stomatal conductance of soybean grown at future CO₂ and ozone concentrations for three years under fully open air field conditions. *Plant, Cell & Environment* **29**: 2077-2090.

Bremer D.J., Ham J.M., Owensby C.E. (1996) Effect of elevated atmospheric carbon dioxide and open top chambers on transpiration in a tall grass prairie. *Journal of Environmental Quality* **20**: 691-701.

Christensen J.H., Christensen O.B. (2003) Climate modelling: Severe summertime flooding in Europe. *Nature* **421**: 805-806.

Ciais P., Reichstein M., Viovy N., Granier A., Ogée J., Allard V., Aubinet M., Buchmann N., Bernhofer Chr., Carrara A., Chevallier F., De Noblet N., Friend A. D., Friedlingstein P., Grünwald T., Heinesch B., Keronen P., Knohl A., Krinner G., Loustau D., Manca G., Matteucci G., Miglietta F., Ourcival J. M., Papale D., Pilegaard K., Rambal S., Seufert G., Soussana J. F., Sanz M. J., Schulze E. D., Vesala T., Valentini R. (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature* **437**: 529-533.

Classens A.T., Langeley J.A. (2005) Data-model integration is not magic: Modelling ecosystem responses to global change: Techniques and recent advances. *New Phytologist* **166**: 367-370

Cleland E.E., Chiariello N.R., Loarie S.R., Mooney H.A., Field C.B. (2006) Diverse responses of phenology to global changes in a grassland ecosystem. *Philosophical Transactions of the National academy of Science* **103**: 13740-13744.

Curtis P.S. (1996) A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. *Plant, Cell & Environment* **19**: 127-137.

Curtis P.S., Wang X. (1998) A meta analysis of elevated CO₂ effects on woody plant mass, form and physiology. *Oecologia* **113**: 299-313.

Dermody O. (2006) Mucking through multifactor experiments; design and analysis of multifactor studies in global change research. *New Phytologist* **172**: 589-591.

DMI.dk. Danish Meteorological Institute homepage:
http://www.dmi.dk/dmi/index/danmark/meteorologiske_ekstremer_i_danmark.htm

Drake B., Gonzalez-Meler M., Long S. (1997) More efficient plants: a consequence of rising atmospheric CO₂? *Annual Review of Plant Physiology and Plant Molecular Biology* **48**: 609-639.

Dubois J.-J.B., Fiscus E.L., Booker F.L., Flowers M.D., Reid C.D. (2007) Optimizing the statistical estimation of the parameters of the Farquhar-von Caemmerer-Berry model of photosynthesis. *New Phytologist* **176**: 402-414.

Dukes J.S., Chiariello N.R., Cleland E.E., Moore L.A., Shaw M.R., Thayer S., Tobeck T., Mooney H.A., Field C.B. (2005) Responses of grassland production to single and multiple global environmental changes. *PLoS Biology* **3**: 1829-1837.

Ellsworth D.S., Reich P.B., Naumburg E.S., Koch G.W., Kubiske M.E., Smith S.D. (2004) Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to

elevated pCO₂ across four free-air CO₂ enrichment experiments in forest, grassland, and dessert. *Global Change Biology* **10**: 2121-2138.

Farquhar G.D., von Caemmerer S., Berry J.A. (1980) A biochemical model of photosynthetic CO₂ carbon dioxide assimilation in leaves of C₃ carbon pathway species. *Planta* **149**: 78-90.

Field C.B., Lund C.P., Chiariello N.R., Mortimer B.E. (1997) CO₂ effects on the water budget of grassland microcosm communities. *Global Change Biology* **3**: 197–206.

Finzi A.C., Allen A.S., Ellsworth D.S., DeLucia E.H., Schlesinger W.H. (2001) Litter production, chemistry and decomposition following two years of free-air CO₂ enrichment. *Ecology* **82**: 470–484.

Finzi A.C., DeLucia E.H., Hamilton J.G., Richter D.D., Schlesinger W.H. (2002) The nitrogen budget of a pine forest under free air CO₂ enrichment. *Oecologia* **132**: 567–578.

Flexas J., Medrano H. (2002) Drought-inhibition of Photosynthesis in C₃ plants: Stomatal and Non-stomatal limitations revisited. *Annals of Botany* **89**: 183-189.

Foggo M.N., Warrington I.J. (1989) Light and vegetative growth in the grasses *Deschampsia flexuosa* (L.) and *Festuga ovina* L. *Functional Ecology* **3**: 439-445.

Foggo M.N. (1989) Vegetative responses of *Deschampsia flexuosa* (L.) Trin. (Poaceae) seedlings to nitrogen supply and photosynthetically active radiation. *Functional Ecology* **3**: 337-343.

Fredeen A.L., Randerson J.T., Holbrook N.M., Field C.B. (1997) Elevated atmospheric CO₂ increases water availability in a water limited grassland ecosystem. *Journal of American Water Resource Association* **33**: 1033–1039.

Gifford R.M. (1992) Interaction of carbon dioxide with growth-limiting environmental factors in vegetation productivity: implications for the global carbon cycle. *Advances in Bioclimatology* **1**: 24–58.

de Graaff M.A., van Groeningen K.J., Six J., Hungate B., van Kessel C. (2006) Interactions between plant growth and soil nutrient cycling under elevated CO₂. *Global Change Biology* **12**: 2077-2091.

Hamerlynck E.P., McAllister C.A., Knapp A.K., Ham J.M., Owensby C.E. (1997) Photosynthetic gas exchange and water relation responses of three tallgrass prairie species to elevated carbon dioxide and moderate drought. *International Journal of Plant Science* **158**: 608–616.

Harte J., Shaw R. (1995) Shifting dominance within a montane vegetation community: results of a climate warming experiment. *Science* **267**: 876-880.

Heimann M., Reichstein M. (2008) Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature* **451**: 289-292.

Hungate B.A., Holland E.A., Jackson R.B., Chapin III F.S., Mooney H.A., Field C.B. (1997) The fate of carbon in grasslands under carbon dioxide enrichment. *Nature* **388**: 576-579.

Hungate B.A., Johnson D.W., Dijkstra P., Hymus G., Stiling P., Megonigal J.P., Pagel A.L., Moan J.L., Day F., Li J., Hinkle C.R., Drake B.G. (2006) Nitrogen cycling during seven years of atmospheric CO₂ enrichment in a scrub oak woodland. *Ecology* **87**: 26-40.

IPCC (2007) Climatic change 2007: The Physical Science Basis. Intergovernmental panel on Climate Change, Geneva.

Jentsch A., Kreylling J., Boettcher-Treschow J., Beierkuhnlein C. (2008) Beyond gradual warming: extreme weather events alter flower phenology of European grassland and heath species. *Global Change Biology* **14**: 1-13.

Karnosky D.F., Mankovska B., Percy K., Dickson R.E., Podila G.K., Sôber J., Noormets A., Hendrey G., Coleman M.D., Kubiske M., Pregitzer K.S., Isebrands J.G. (1999) Effects of tropospheric O₃ on trembling aspen and interaction with CO₂: Results from an O₃-gradient and a FACE experiment. *Water, Air and Soil Pollution* **116**: 311-322.

Kelliher F.M., Leuning R., Schulze E.D. (1993) Evaporation and canopy characteristics of coniferous forests and grasslands. *Oecologia* **95**: 153-163.

King, J.S., Pregitzer, K.S., Zak, D.R., Sober, J., Isebrands, J.G., Dickson, R.E., Hendrey, G.R. and Karnosky, D.F. (2001) Fine-root biomass and fluxes of soil carbon in young stands of paper birch and trembling aspen as affected by elevated atmospheric CO₂ and tropospheric O₃. *Oecologia* **128**: 237-250.

Knapp A.K., Hamerlynck E.P., Ham J.M., Owensby C.E. (1996) Responses in stomatal conductance to elevated CO₂ in 12 grassland species that differ in growth form. *Vegetatio* **125**: 31-41.

Knapp K.A., Fay P.A., Blair J.M., Collins S.L., Smidth M.D., Carlisle J.D., Harper C.W., Danner B.T., Lett M.S., McCarron J.K. (2002) Rainfall variability, carbon cycling, and Plant species diversity in a mesic grassland. *Science* **298**: 2202-2205.

Koch G.W., Mooney H.A. (1996) Response of terrestrial ecosystems to elevated CO₂: a synthesis and summary. In: Koch GW, Mooney HA (eds) Carbon dioxide and terrestrial ecosystems. Academic Press, San Diego, pp 415-429

Körner C. (2000) Biosphere responses to CO₂ enrichment. *Ecological Applications* **10**: 1590–1619.

Körner C., Asshoff R., Bignucolo O., Hättenschwiler S., Keel S.G., Peláez-Riedl S., Pepin S., Siegwolf R.T.W., Zotz G. (2005) Carbon flux and growth in mature deciduous forest trees exposed to elevated CO₂. *Science* **309**: 1360 – 1362.

Körner C. (2006) Plant CO₂ responses: An issue of definition, time and resource supply. *New Phytologist* **172**: 393-411.

Lauber I.R. Körner C. (1997) *In situ* stomatal response to long term CO₂ enrichment in calcareous grassland plants. *Acta Oecologia* **18**: 221-229.

Leuzinger S., Körner C. (2007) Water savings in mature deciduous forest trees under elevated CO₂. *Global Change Biology* **13**: 2498–2508.

Liberloo M., Tulva I., Raïm O., Kull O., Ceulemans (2007) Photosynthetic stimulation under long-term CO₂ enrichment and fertilization is sustained across a closed *Populus* canopy profile (EUROFACE). *New Phytologist* **173**: 537-549.

Long S.P., Drake B.G. (1992) Photosynthetic CO₂ assimilation and rising CO₂ concentrations. In Crop photosynthesis spatial and temporal determinants (eds Baker N.R. and Thomas H.), pp69-103, Elsevier, Amsterdam, The Netherlands.

Long S.P., Ainsworth E.A., Rogers A., Ort D.R. (2004) Rising atmospheric carbon dioxide: plants FACE the future. *Annual review of Plant Biology* **55**: 591-628.

Long S.P., Ainsworth E.A., Bernacchi E.A., Davey P.A., Hymus G.J., Leakey A.D.B., Morgan P.B., Osborne C.P. (2006a) Long term responses of photosynthesis and stomata to elevated CO₂ in managed systems. In Managed Ecosystems and CO₂. Case studies, Processes and Perspectives (eds Nösberger J., Long S.P., Norby R.J., Stitt M., Hendry G.R., Blum H), pp 253-270, Springer-Verlag, Heidelberg, Germany.

Long S.P., Ainsworth E.A., Leakey A.B.D., Nosberger J. Ort D.R. (2006b) Food for thought: lower than expected crop yield stimulation with rising CO₂ concentrations. *Science* **312**: 1918-1921.

Lou Y., Wan S., Hui D., Wallace L.L. (2001) Acclimatization of soil respiration to warming in a tall grass prairie. *Nature* **413**: 622-625.

Lou Y., Su B., Currie W.S., Dukes J.S., Finzi A., Hartwig U., Hungate B., McMurtrie R.E., Oreon R., Parton W.J., Pataki D.E, Shaw M.R., Zak D.R, Field C.B. (2004) Progressive Nitrogen Limitation of Ecosystem Responses to Rising Atmospheric Carbon Dioxide. *Bioscience* **54**: 731-739.

Lou Y. (2007) Terrestrial carbon cycle feedback to climate warming. *Annual Review of Ecology, Evolution and Systematics* **38**: 683-712.

Medlyn B.E., Barton C.V.M., Broadmeadow M.S.J., Ceuleman R., De Angelis P., Forstreuter M., Freeman M., Jackson S.B., Kellomaki S., Laitat E., Rey A., Roberntz P., Sigurdsson B.D., Strassmeyer J., Wang K., Curtis P.S., Jarvis (2001) Stomatal conductance of forest species after long-term exposure to elevated CO₂ concentration: a synthesis. *New Phytologist* **149**: 247–264.

Menzel A., Fabian P. (1999) Growing season extended in Europe. *Nature* **397**: 659.

Menzel A., Sparks T.H., Estrella N., Koch E., Aasa A., Ahas R., Alm-Kübler K., Bissolli P., Braslavska O., Briede A., Chielewski F.M., Crepensek Z., Curnel Y., Dahl Å., Defila C., Donnelly A., Filella Y., Jateczak K., Måge F., Mestre A., Nordli Ø., Penuelas J., Pirinen P., Remosova V., Scheifinger H., Striz M., Susnik A., van Vliet A.J.H., Wielgolaski F.-E., Zach S. and Züst A. (2006) European phenological response to climate change matches the warming pattern. *Global Change Biology* **12**: 1969-1976.

Miglietta F., Peressotti A., Vaccari F.P., Zaldei A., deAngelis P. & Scarascia-Mugnozza, G. (2001) Free-air CO₂ enrichment (FACE) of a poplar plantation: the POPFACE fumigation system. *New Phytologist* **150**: 465–476.

Mikkelsen T.N., Beier C., Jonasson S., Holmstrup M., Schmidt I.K., Ambus P., Pilegaard K., Michelsen A., Albert K.R., Andresen L.C., Arndal M.F., Bruun N., Christensen S., Danbæk S., Gundersen P., Jørgensen P., Kongstad J., Maraldo K., Primé A., Riis-Nielsen T., Ro-Poulsen H., Stevnbak K., Selsted M.B., Sørensen P., Larsen K.S., Carter M.S., Martinussen T., Miglietta F., Sverdrup H. (2008) Experimental design of multifactor climate change experiments with elevated CO₂, warming and drought – the CLIMAITE project. *Functional Ecology* **22**: 185-195.

Moore B.D., Cheng S-H., Sims D., Seemann J.R. (1999) The biochemical and molecular basis for photosynthetic acclimation to elevated atmospheric CO₂. *Plant Cell & Environment* **22**: 567-582.

Morison J.I.L., Gifford R.M. (1984) Plant growth and water use with limited water supply in high CO₂ concentrations. II. Plant dry weight, partitioning and water use efficiency. *Australian Journal of Plant Physiology* **11**: 375–384.

Morgan J.A., Pataki D.E., Körner C., Clark H., Del Grosso S.J., Grünzweig J.M., Knapp A.K., Mosier A.R., Newton P.C.D., Niklaus P.A., Nippert J.B., Nowak R.S., Parton W.J., Polley H.W., Shaw M.R. (2004) Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO₂. *Oecologia* **140**: 11–25.

Niklaus P.A., Spinnler D., Körner C. (1998) Soil moisture dynamics of calcareous grassland under elevated CO₂. *Oecologia* **117**: 201–208.

Norby R.J., Lou Y. (2004) Evaluating ecosystem responses to rising atmospheric CO₂ and global warming in a multi-factor world. *New Phytologist* **162**: 281-293.

Nowak R.S., Ellsworth D.S., Smith S.D. (2004) Functional responses of plants to elevated atmospheric CO₂ – do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytologist* **162**: 253-280.

Oren R., Ellsworth D.S., Johnsen K.H., Phillips N., Ewers B.E., Maier C., Schäfer K.V.R., McCarthy H., Hendry G., McNulty S.G., Katul G.C. (2001) Soil fertility limits carbon sequestration by forest ecosystems in a CO₂ enriched atmosphere. *Nature* **411**: 469-472.

Parry M.A.J., Andralojc P.J., Mitchell R.A.C., Madgwick P.J., Keys A.J. (2003) Manipulation of Rubisco: the amount, activity, function and regulation. *Journal of Experimental Botany* **54**:1321-1333.

Penuelas J. and Filella I. (2001) Responses to a warming world. *Science* **294**:793-795.

Peñuelas J., Prieto P., Beier C., Cesaraccio C., de Angelis P., de Dato G., Emmett B.A., Estiarte M., Garadnai J., Gorissen A., Lang E.K., Kröel-Dulay G., Llorens L., Pellizzaro G., Riis-Nielsen T., Schmidt I.K, Sirca C., Sowerby A., Spano D., Tietma A. (2007) Response of plant species richness and primary productivity in shrublands along a north-south gradient in Europe to seven years of experimental warming and drought: reductions in primary productivity in the heat and drought year of 2003. *Global Change Biology* **13**: 2563-2581.

Reich P.B., Hobbie S.E., Lee T., Ellsworth D.S., West J.B., Tilman D., Knops J.M.H., Naeem S., Trost J. (2006) Nitrogen limitation constrains sustainability of ecosystem response to CO₂. *Nature* **440**: 922-925.

Robredo A., Pérez-López U., Sainz de la Maza H., González-Moro B., Lacuesta M., Mena-Petite A., Munoz-Rueda A. (2007) Elevated CO₂ alleviates the impact of drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis. *Environmental and Experimental Botany* **59**: 252-263.

Rogers A., Fischer B.U., Bryant J., Frehner M., Blum H., Raines C.A., Long S.P. (1998) Acclimation of photosynthesis to elevated CO₂ under low-nitrogen nutrition is affected by the capacity for assimilate utilization. Perennial ryegrass under free air CO₂ enrichment. *Plant Physiology* **118**: 683-689.

Rogers A., Allen D.J., Davey P.A., Morgan P.B., Ainsworth E.A., Bernacchi C.J., Cornic G., Dermody O., Dohleman F.G., Heaton E.A., Mahoney J., Zhu X.G., Delucia E.H., Ort D.R., Long S.P. (2004) Leaf photosynthesis and carbohydrate dynamics of soybeans grown throughout their life-cycle under Free-Air Carbon dioxide enrichment. *Plant Cell & Environment* **27**: 449-458.

Rogers A., Ainsworth E.A. (2006) The response of foliar carbohydrates to elevated carbon dioxide concentration. In *Managed Ecosystems and CO₂. Case Studies, Processes and Perspectives* (eds. Nösberger J., Long S.P., Norby R.J., Stitt M., Hendrey G.R., Blum H.) pp 293-308. Springer Verlag, Heidelberg, Germany.

Rogers A., Ainsworth E.A., Kamman C. (2006) Perspectives of the future of free air CO₂ enrichment studies. In *Managed Ecosystems and CO₂. Case studies, Processes and Perspectives* (eds Nösberger J., Long S.P., Norby R.J., Stitte M., Hendrey G.H., Blum H.), pp 431-450, Springer-Verlag, Heidelberg, Germany.

Rustad L., Campbell J., Marion G., Norby R., Mitchell M., Hartley A., Cornelissen J., Gurevitch J., GCTE-NEWS. (2001) A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* **126**: 543–562.

Rustad L.E. (2006) From transient to steady state response of ecosystems to atmospheric CO₂-enrichment and global climate change: Conceptual challenges and need for an integrated research. *Plant Ecology* **182**: 43-62.

Rustad L.E. (2008) The response of terrestrial ecosystems to global climate change: Towards an integrated approach. *Science of the total environment* **404**: 222-235.

Sage R.F., Kubien D.S. (2007) The temperature response of C3 and C4 photosynthesis. *Plant, Cell & Environment* **30**: 1086-1106.

Shaver G.R., Canadell J., Chapin F.S. III, Gurevitch J., Harte J., Henry G., Ineson P., Jonasson S., Melillo J, Pitelka L., Rustad L. (2000) Global warming and terrestrial ecosystems: a conceptual framework for analysis. *Bioscience* **50**: 871-882.

Shaw M.R., Zavaleta E.S., Chiariello N.R., Cleland E.E., Mooney H.A., Field C.B. (2002) Grassland responses to global environmental changes suppressed by elevated CO₂. *Science* **298**: 1987-1990.

Springer J.C., Ward J.K. (2007) Flowering time and CO₂. *New Phytologist* **176**: 243-255.

Teng N., Wang J., Chen T., Wu X., Wang Y., Lin J. (2006) Elevated CO₂ induces physiological, biochemical and structural changes in leaves of *Arabidopsis thaliana*. *New Phytologist* **172**: 378-378.

Turnbull M.H., Murthy R., Griffin K.L (2002) The relative impacts of daytime and night time warming on photosynthetic capacity in *Populus deltoides*. *Plant Cell & Environment* **25**: 1729-1737.

Turnbull M.H., Tissue D.T., Murthy R., Wang X., Sparrow A.D., Griffin K.L. (2004) Nocturnal warming increases photosynthesis at elevated CO₂ partial pressure in *Populus deltoides*. *New Phytologist* **161**: 819-826.

Volk M., Niklaus P.A., Körner C. (2000) Soil moisture effects determine CO₂ responses of grassland species *Oecologia* **125**: 380–388.

Wall G.W., Brooks T.J., Adam N.R., Cousins A., Triggs J., Kimball B.A., Pinter P.J. Jr, LaMorte R.L., Ottman M.J., Leavitt S.W., Matthias A.D., Williams D.G., Webber A.N. (2001) Leaf photosynthesis and water relations of grain sorghum grown in free-air CO₂ enrichment (FACE) and water stress. *New Phytologist* **152**: 231-248

Walther G-R., Post E., Convey P., Menzel A., Parmesan C., Beebee T. J. C., Fromentin J-M., Hoegh-Guldberg O., Bairlein F. (2002) Ecological responses to recent climate change. *Nature* **416**: 389-395.

Wan S., Hui., Wallace., Lou Y. (2005) Direct and indirect effects of experimental warming on ecosystem carbon processes in a tall grass prairie. *Global Biogeochemical Cycles* **19**: GB2014. Doi:10.1029/2004GB002315.

Figure 1: Seasonal variation in climate parameters. Shown are in (upper) the daily averages of soil water content in control plots in 0-20 cm and 0-60 cm depth and daily accumulated precipitation, (middle) daily averages of air temperature in 2m height and (lower) photosynthetically active radiation (PAR).

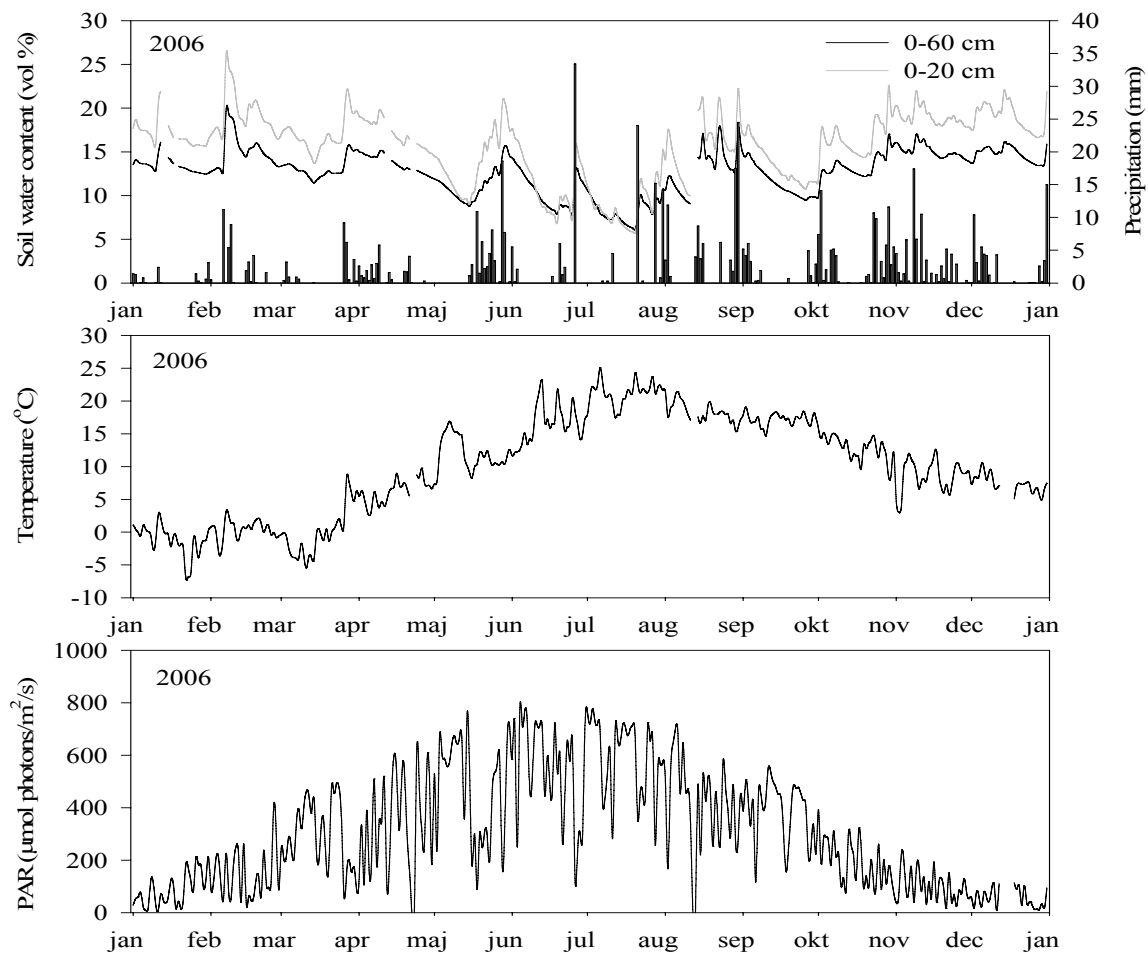


Figure 2: Soil water content in 0-20 cm depth, SWC, during each gas-exchange campaign. Shown are the mean \pm standard errors. Treatments are (A) un-manipulated control, (T) passive nighttime warming, (D) experimental drought, active in July- early August, (TD) warming and drought combined, (CO₂) elevated CO₂, (TCO₂) warming and elevated CO₂ combined, (DCO₂) drought and elevated CO₂ combined, (TDCO₂) warming, drought and elevated CO₂ combined. Statistically significant factors and interactions are shown with treatment letters and levels of significance with *** if $p < 0.0001$, ** if $p < 0.001$, * if $p < 0.05$ and † if $p < 0.10$.

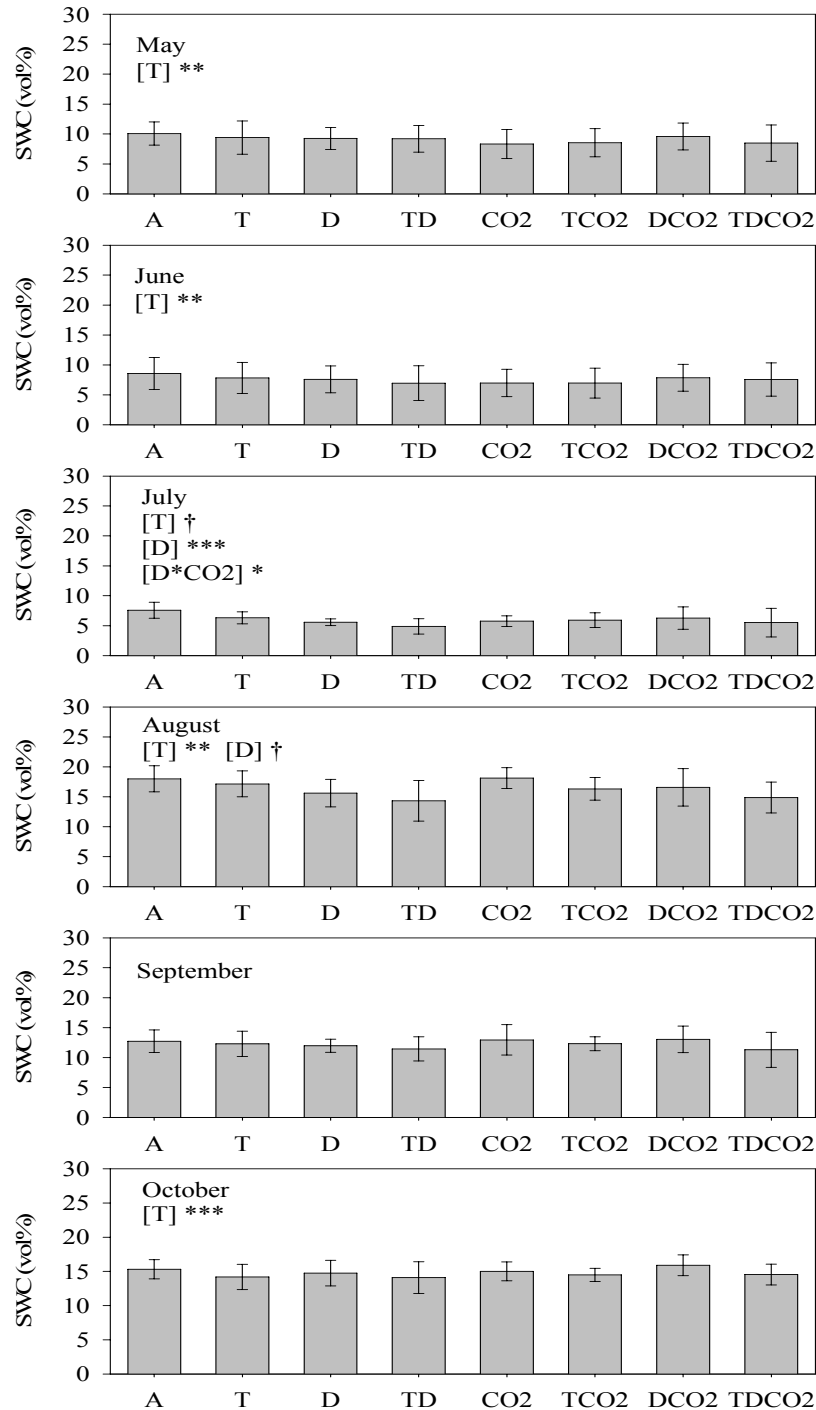


Figure 3: Light saturated net photosynthesis, P_n . Shown are the mean \pm standard errors. Treatments abbreviations and statistics as in figure 2.

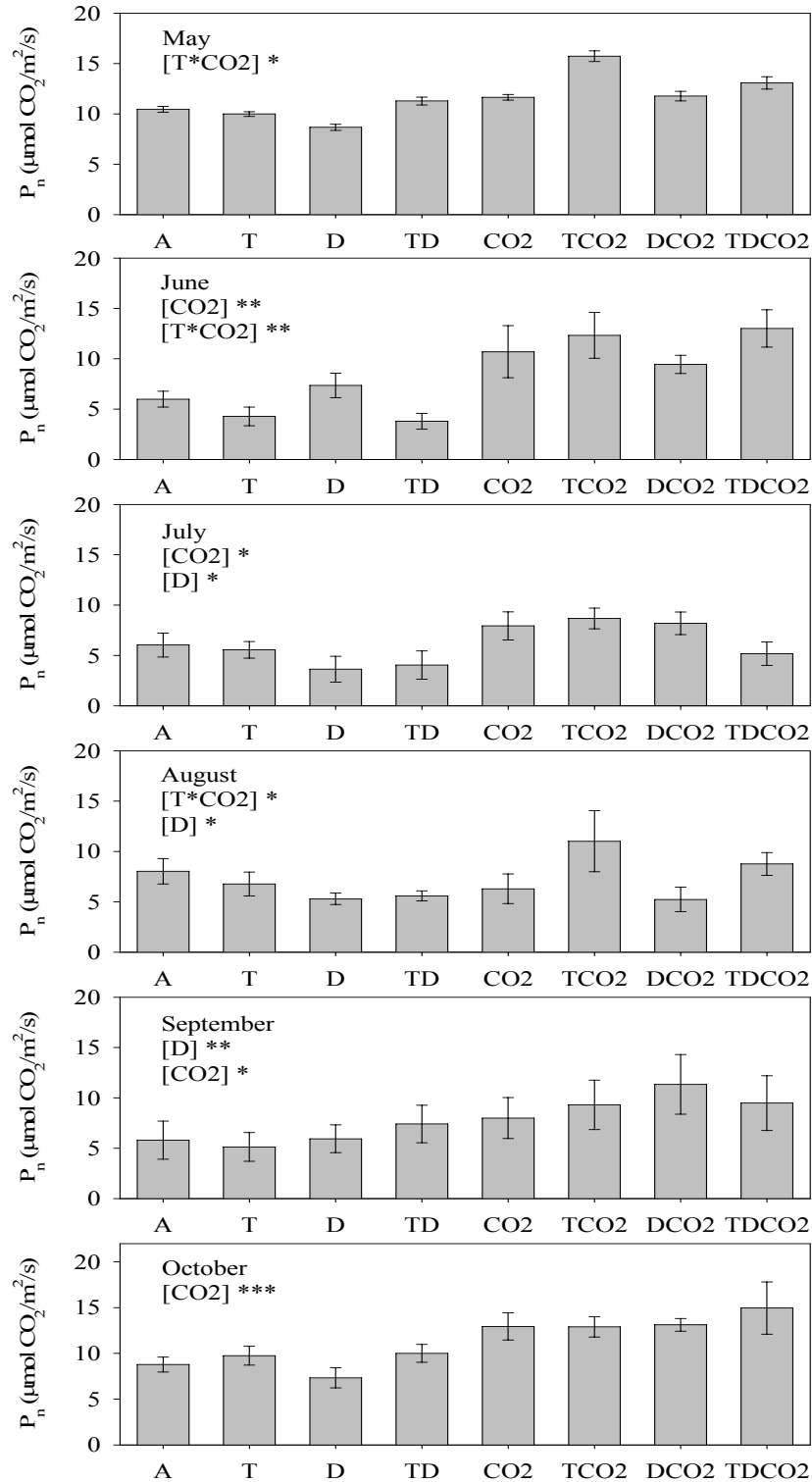


Figure 4: Leaf stomatal conductance, g_s . Shown are the mean \pm standard errors. Treatments abbreviations and statistics as in figure 2.

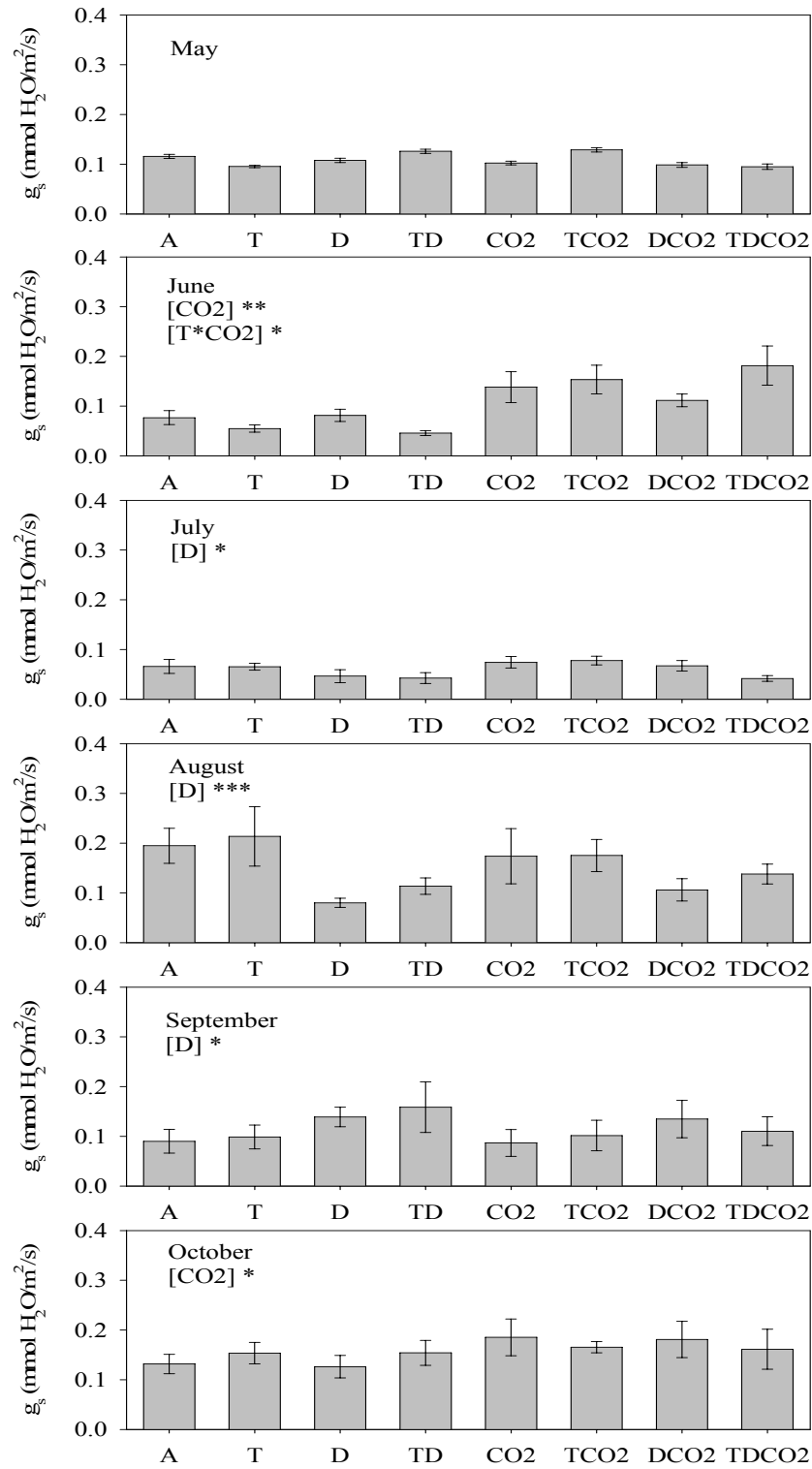


Figure 5: Photosynthetic water use efficiency, WUE. Shown are the mean \pm standard errors. Treatments abbreviations and statistics as in figure 2.

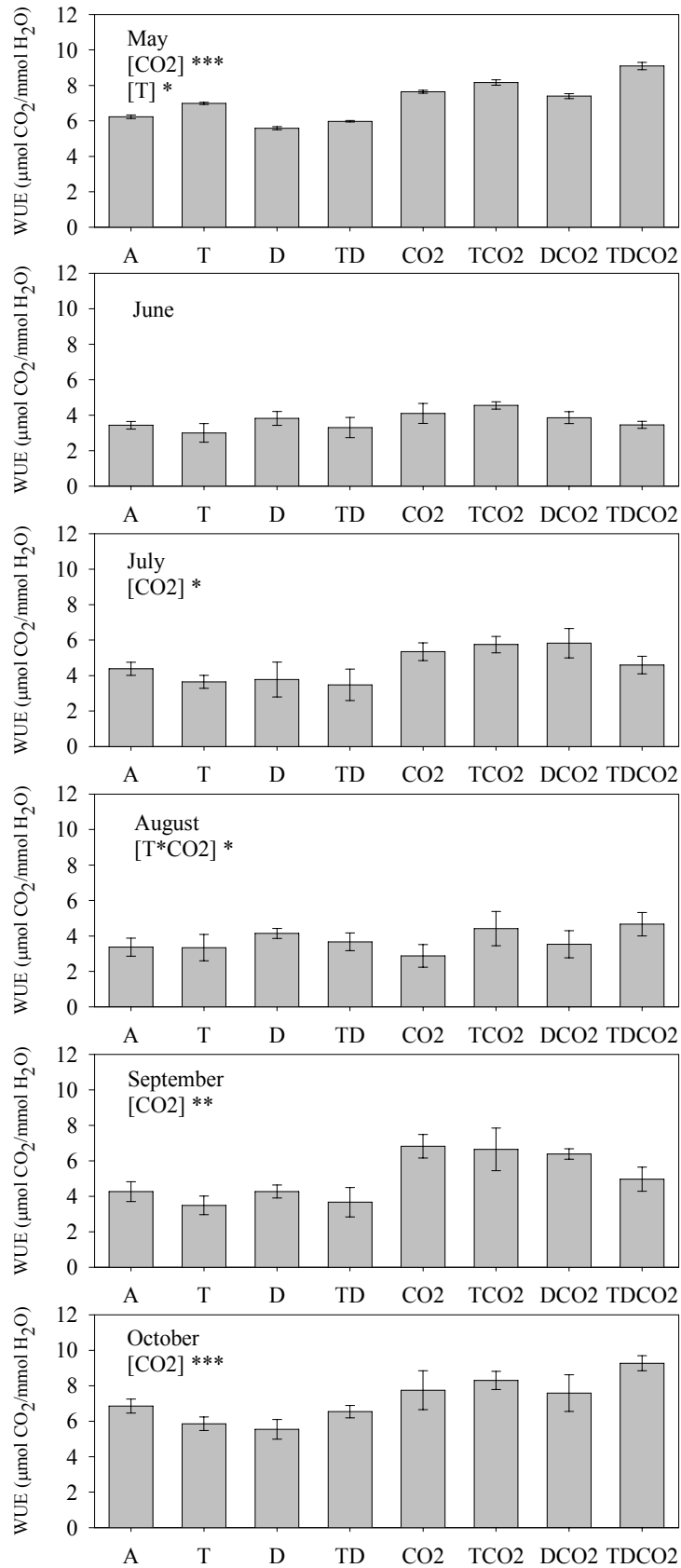


Figure 6: Leaf carbon to nitrogen ratio, C/N. Shown are the mean \pm standard errors. Treatments abbreviations and statistics as in figure 2.

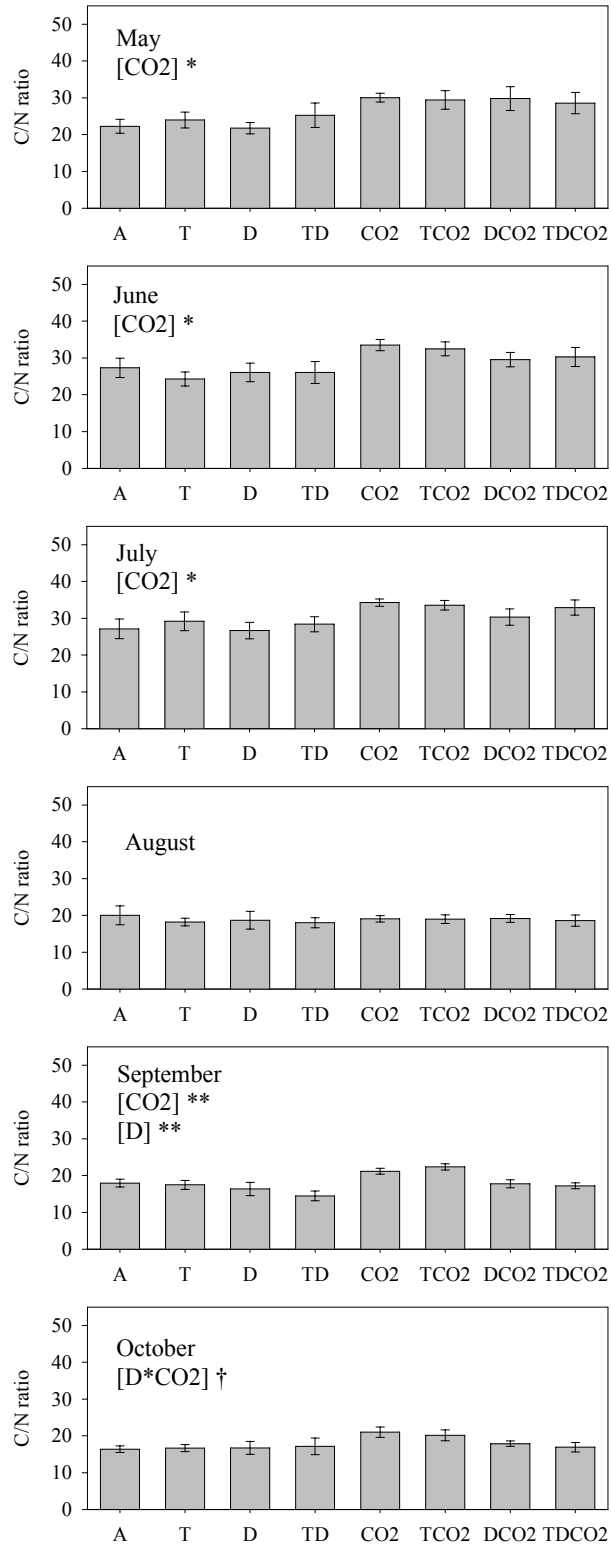
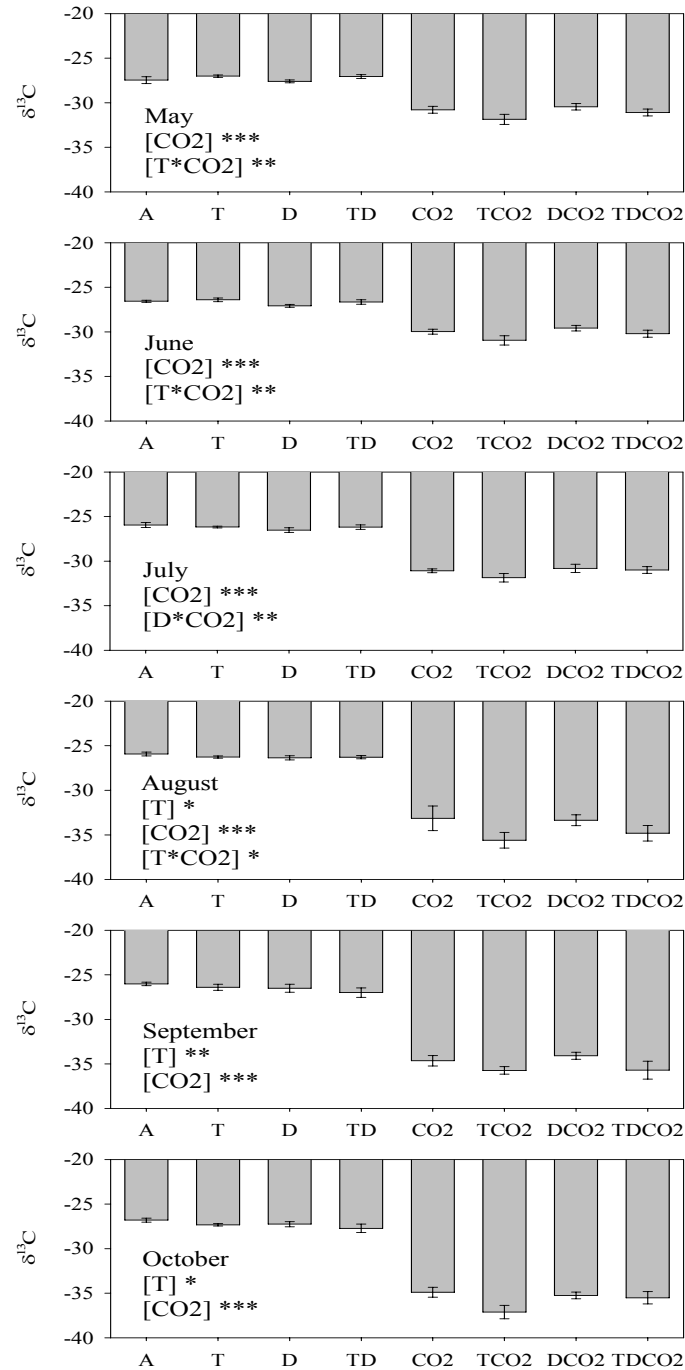


Figure 7: Natural abundance of leaf ^{13}C , $\delta^{13}\text{C}$. Shown are the mean \pm standard errors. Treatments abbreviations and statistics as in figure 2. Note that the industrial CO_2 gas dosed in the FACE plots had a lower $\delta^{13}\text{C}$ signature (c. -28.1 $\delta^{13}\text{C}$ as opposed to -8.0 in ambient air) causing the significantly lower $\delta^{13}\text{C}$ values in leaves in elevated CO_2 treatment.



Paper III

Prolonged growing season and complex water relations in interactions of CO₂, warming and drought on *Calluna vulgaris* in a temperate heath.

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Manuscript

Prolonged growing season and complex water relations in interactions of CO₂, warming and drought on *Calluna vulgaris* in a temperate heath.

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List of abbreviations:

leaf carbon concentration (C), leaf carbon to nitrogen ratio (C/N-ratio), carbon isotope ratio (¹³C), Stomatal conductance (g_s), Maximal velocity of RubP regeneration (J_{max}), leaf nitrogen concentration (N), light saturated net photosynthesis (P_n), photosynthetic nitrogen use efficiency (PNUE), maximal velocity of Rubisco carboxylation (V_{cmax}), water use efficiency (WUE), soil water content (SWC), plant xylem water potential (PWP), passive night time warming (T), experimental drought (D), experimental elevated CO₂ (CO₂), specific leaf area (SLA), growing degree days (GDD), free air carbon enrichment (FACE).

Keywords:

CLIMATE, multi factor experiment, net photosynthesis, heath ecosystem, FACE, J_{max}, V_{cmax}, climate change, gas exchange, photosynthesis, stomatal conductance.

Abstract

Increased atmospheric CO₂, warming and changed precipitation patterns impact ecosystem structure and processes. The potential for non-additive effects when these factors are combined is adding complexity and challenges to our current understanding. We therefore investigated the impact of elevated CO₂ (FACE), periodic drought (automatized water excluding curtains) and warming (nighttime IR-reflective curtains) on photosynthesis and leaf characteristics in the multi-factorial experiment, on the evergreen dwarf shrub *Calluna vulgaris* in a temperate heath ecosystem. Warming and elevated CO₂ synergistically increased net photosynthesis in early spring and late autumn. This increased leaf C/N ratio and decreased $\delta^{13}\text{C}$ indicating pronounced carbon uptake by combining warming and elevated CO₂. Elevated CO₂ stimulated photosynthesis and water use efficiency. Drought decreased plant water potential, stomatal conductance and net photosynthesis. The responses during drought and after rewetting were complex. For instance, when close to the wilting point, warming and drought synergistically decreased plant water potential and stomatal conductance, but there were no impact on net photosynthesis. At this time elevated CO₂ stimulated photosynthesis, probably due to improved water relations. After rewetting net photosynthesis was decreased in drought and warming plots, but increased in elevated CO₂ plots. However, with elevated CO₂ and warming combined non-additive decreased photosynthesis were seen. Our results demonstrate the clear control of water relations on net photosynthesis stimulation in elevated CO₂. In the combined treatment, warming and enhanced CO₂ and drought, that best represents the predicted future climate, the productivity increased almost at all time an growing season was expanded.

Introduction

The climate is changing and the anthropogenic forcing have been thoroughly documented (IPCC 2001, 2007). The level of atmospheric CO₂ has increased from a preindustrial level of 270 ppm to current values around 380 ppm, and is expected to increase to around c. 700 ppm (IPCC 2001). Warming will be more pronounced during nighttime and temperature increases of 1.4-5.8°C are expected during the next 100 years (IPCC 2001). Precipitation changes with prolonged summer droughts, heavy precipitation events and higher frequency of extremes are also expected (IPCC 2007). This has attracted focus on the ecosystem responses to climatic changes and also on the ecosystem feedbacks to climate and large efforts are invested in understanding these concerted and complex impacts (Heimann and Reichstein 2008, Rustad 2006, 2008). One tool is the analysis of responses in multi-factor experimental manipulations studies (Beier *et al.* 2004, Classen and Langley 2005, Heimann and Reichstein 2008, Norby and Lou 2004). Despite substantial knowledge of the impact of single factors as e.g. elevated CO₂ (e.g. Ainsworth and Long 2005, Ainsworth and Rogers 2007, Körner 2000, Drake *et al.* 1997, Hungate *et al.* 1997a), drought (e.g. Chaves 1991, Schultze 1986, Yordanov *et al.* 2000) and warming (e.g. Lou 2007, Penuelas and Filella 2001), multi-factor studies are necessary as the interactions between drivers may act synergistically or antagonistically upon dependent variables and thereby yield surprising results (Shaw *et al.* 2002, Henry *et al.* 2005, Norby *et al.* 2007, Lou *et al.* 2008). The multi-factorial approach may facilitate in-depth investigations of the causal mechanisms involved, gain new insight and push understanding further forward (Heimann and Reichstein 2008, Rustad 2008). In a

temperate heath ecosystem we therefore applied manipulations with elevated CO₂ (FACE), passive night time warming (nighttime IR-curtains) and summer drought (curtains excluding precipitation) closely adjusted to the scenario for climatic conditions in Denmark in 2075 (Mikkelsen *et al.* 2008). We focused on the strength of drivers and how their impact changed during growing season on the photosynthetic and leaf characteristics of the evergreen dwarf shrub *Calluna vulgaris*. *Calluna vulgaris* has xeromorphic traits as deep roots and small in-rolled leaves surfaced with wax and trichomes sheltering the stomates and it tolerates very low water availability (Gordon *et al.* 1999 and Llorens *et al.* 2004).

The photosynthetic carbon uptake depends on the resources available, e.g. irradiance, soil moisture and soil nutrient availability (Curtis and Wang 1998) and it is likely that a changing sequence of the factors limiting carbon sink uptake may appear as the ecosystems adjust to a new steady state. In elevated CO₂ responses may be modulated by precipitation patterns and periodical water shortage (Heisler and Weltzin 2006, Körner 2000, Knapp *et al.* 2002, Volk *et al.* 2002), particularly in grasslands (Morgan *et al.* 2004, Körner 2006, Robredo *et al.* 2007). Decreased responsiveness of photosynthetic stimulation in elevated CO₂ with age have been reported in closed forest stands (summarized in Körner 2006) and similar responses may be of importance for dwarf shrubs in the end of their natural life cycle. In the long term nutrient availability will be of increasing importance for sustained productivity in elevated CO₂ (Lou *et al.* 2004, Menge and Field 2007, Oren *et al.* 2001, Reich *et al.* 2006).

Warming has the ability to advance growth season (Menzel and Fabian 1999, Menzel *et al.* 2006, Wan *et al.* 2004), shift phenology (Penuelas and Filella 2001, Cleland

et al. 2006, Jentsch *et al.* 2008) and increase carbon uptake due to increased length of the growing season (White *et al.* 1999, Piao *et al.* 2007). However, warming may also stimulate soil microbes that decompose litter, returning a comparable amount of CO₂ to the atmosphere (Piao *et al.* 2007).

Drought clearly decreases stomatal conductance and net photosynthesis (Chaves 1991, Flexas and Medrano 2002, Schulze 1986, Yordanov *et al.* 2000). The impact of precipitation variability and frequency is of importance for productivity (Heisler and Weltzin 2006, Knapp *et al.* 2002) and severe drought events can substantially decrease productivity (Ciais *et al.* 2005). In our study the experimental drought extended the natural summer drought periodically and prolonged the time when plants were close to the wilting point. Our experimental setup allowed us to investigate the counteracting negative impact of drought and beneficial impact of elevated CO₂ on photosynthesis characteristics at a range of SWC conditions. When combined with the effects of warming the water relations, e.g. via season length increments, become more complicated and provides basis for new insight. Because the plant responses to climatic change may vary during growing season we therefore investigated the responses through monthly campaigns. Here we report the impact on photosynthesis and leaf characteristics of passive night time warming [T], drought [D], elevated CO₂ [CO₂] and their interactions. Efforts to demonstrate the strong linkages cross scales of responses variables (photosynthesis, stomatal conductance, leaf C/N, $\delta^{13}\text{C}$, plant water potential, specific leaf area and green-to-brown shoot ratios) were included to consolidate our findings of the responses of the dwarf shrub in this heathland ecosystem.

We hypothesized that:

- Warming advances the spring and also extends the growing season in the autumn
- Experimental drought decreases plant water potential, stomatal conductance and photosynthesis
- Elevated CO₂ increases photosynthesis and decreases stomatal conductance
- Elevated CO₂ and warming in combination increases plant carbon uptake
- Elevated CO₂ mitigates the negative impacts of experimental drought
- Warming and experimental drought in combination decreases photosynthesis
- Increased productivity increases the leaf C/N ratio
- Improved water relations and carbon uptake in combination decreases $\delta^{13}\text{C}$

Materials and Methods

Site and experimental setup

The vegetation is a dry heath land ecosystem on sandy soil in North Zealand, Denmark, dominated by the evergreen dwarf shrub heather (*Calluna vulgaris* L.) and hairgrass (*Deschampsia flexuosa* L.). The plant cover was subjected to the following experimental treatments: Un-manipulated control [A], elevated CO₂ (CO₂), passive nighttime warming (T), summer drought (D), and all combinations (TD), (TCO₂), (DCO₂), (TDCO₂) replicated in six blocks in a split-plot design. Each block consisted of two octagons of 8m diameter each divided in four plots. In one octagon CO₂ was elevated to 510 ppm during daylight hours with the FACE technique and in the other octagon CO₂ level was ambient. In each octagon automatized infrared reflective curtains covered one half during night preserving a larger proportion of the daily incoming radiation energy, which increased the night air temperature with 1.4°C in average. The plots perpendicular to the IR-curtains

was periodically covered with a rain-excluding curtain which automatically was activated by rain, thus creating an artificial drought period (Mikkelsen *et al.* 2008). In each experimental plot the temperature was measured in 2 and 5 cm depth and the soil moisture in 0-20 and 0-60 cm depth. Two climatic stations recorded precipitation, air temperature, photosynthetically active radiation, wind directions and relative humidity (Mikkelsen *et al.* 2008). The CO₂ and warming treatments were initiated in 3rd October 2005. The drought treatment started 3rd July 2006 and was continued for 5 weeks until 4th August when soil water content approached c. 5 % water content in the top 20 cm of the soil. For further description of site and experimental setup, see Mikkelsen *et al.* 2008.

Leaf gas exchange

Gas exchange measurements of CO₂ and H₂O fluxes were conducted *in situ* by using two LI-6400's (LI-COR Biosciences, Lincoln, Nebraska, USA) connected to standard 2x3 cm² chambers with LED light source (6400-02B) and were carried out in 2006: May (18-19th); June (13-14th); July (9-10th); August (17-21st); September (14-22nd) and October (13-20th). Only healthy shoots from the top of the *Calluna* plants were selected. Pilot studies led to the following methodology securing highly reproducible measurements: The shoots (one per plot) were flattened between transparent 0.2mm nylon strings suspended in a metal frame with spacing 2.5-4 mm. The position of the shoots was upright and south facing. Leaf cuvettes were then fixed to the samples using flexible rods (Magic Arm 143, Manfrotto, Italy) fastened to the octagon scaffolds. The protocol for CO₂-response curves were optimized and tested and resulted in the following procedure: Samples were acclimated 2-4 minutes at ambient CO₂ (380 ppm), until net photosynthesis

and stomatal conductance were stabilized (± 1 CV over 30 sec). The CO₂ response curves were measured by stepping down [CO₂] from 380 ppm to 300, 200, 100 and 50 ppm, and then taken back to 380 ppm for 4 minutes re-acclimation until initial state was reached again. The [CO₂] was then stepped up to saturation from 380 ppm to 450, 510, 650, 800, 1000, 1200 and 1400 ppm. The measurements were done at saturating light level, 1500 $\mu\text{mol photons/m}^2/\text{s}$, using the LI-COR Auto program 'A/C_i-curve' (Settings: Min 45 and max 60 sec, reference CO₂ stable in 10 sec with CV<1%, C_i stable in 10 sec with CV<1%, IRGA matching performed at each step). Block temperature was held constant at 25°C and sample relative humidity were stabilized at 45-55% during measurements. All measurements were area corrected (see below). Gas-exchange parameters, i.e. light saturated net photosynthesis, P_n, transpiration, T_r, stomatal conductance, g_s and intercellular CO₂ concentration, C_i, were extracted from the CO₂ response curves at CO₂Ref = 380 ppm CO₂ in non-FACE plots and at CO₂Ref = 510 ppm CO₂ in FACE plots. Calculation of maximum rate of Rubisco carboxylation, V_{max} and maximum rate of RubB regeneration, J_{max}, followed the approach of Dubois *et al.* (2007) based on the principles originating from Farquhar *et al.* (1980).

Plant water potential

Plant water potential was measured using the Scholander method (PWSC Research Model 3000, Soil moisture Equipment Corp., Goleta, CA 93117 USA). In each plot 2-4 shoots were randomly sampled from the top of the canopy during the last campaign night in July, August, September and October during 2-5 o'clock (pre-dawn). The sampling

was done one block at a time and measurements were conducted as fast as possible after sampling.

Leaf characteristics: Weight, area, nitrogen, carbon and ^{13}C

The shoots were harvested after each period (see above) and care was taken to ensure that weight and area were determined on exactly the same material as inserted in the leaf cuvette. Immediately after harvest digital pictures were taken of plant material flattened by transparent Plexiglas together with a brick of known area. The photographs were converted to black and white and loaded in a pixel counting program (Bitmap, S. Danbæk, Department of Biology, University of Copenhagen) and the projected shoot areas were determined. Shoots were split into photosynthetic active parts ('green') and the rest ('brown'). Then the fresh weights were determined. The dry weight was determined after oven drying at 80°C. The plant material was analyzed for C and N concentration and ^{13}C natural abundance ($\delta^{13}\text{C}$) with a Eurovector CN analyzer coupled to an Isoprime isotope ratio mass spectrometer. During analysis, the internal reference gas was calibrated against certified standards from the International Atomic Energy Agency, and plant material calibrated against certified standards was used as working standard. Leaf $\delta^{13}\text{C}$ is expected to increase in plant with higher WUE. From dry weights and leaf area the specific leaf area, SLA, were calculated. Leaf water content was calculated from fresh and dry weights.

Statistics

Analyses of variance were made in SAS using the proc mixed to test for effects of the fixed factors: [Time], [T], [D], [CO₂] and their interactions T*D, T*CO₂, D*CO₂, T*D*CO₂. To take into account the split-plot design, the plot was nested within octagon and octagon nested within block and these two terms set as random factors. Pre-treatment data were initially included in the full model as co-variates and handled as a fixed factor. The model were reduced by progressively dropping in-significant fixed factors, starting with the higher order interactions until only significant ($P < 0.05$) or near significant terms ($p < 0.10$) remained. Significance levels are reported as a tendency with † when $p < 0.10$ and as significant with * when $p < 0.05$, ** when $p < 0.001$ and *** when $p < 0.0001$. All data were normal distributed and some parameters were transformed to fulfill the assumption of variance homogeneity. To interpret the direction of the significant responses we compared the least squared means.

Results

Climate and experimental control measurements

Snow melted in late March and in early April the mean daily temperature in 2m height increased above freezing and gradually increased to 25°C in late July. Autumn 2006 were the warmest ever recorded in Denmark (12.2°C, DMI.dk) and mean daily air temperatures were above 11°C in late November (Fig. 1). Warming increased the canopy temperature 1-2°C and there were 33% higher accumulated GDD from 1st April to 15th May and an annual 7% GDD increase in warmed plots compared to controls (Mikkelsen *et al.* 2008), with a GDD criteria assuming a threshold for growth at 5°C (Beier *et al.* 2004). The CO₂ concentrations in the FACE plots were elevated to 510 ppm during

daytime, but not during night, with monthly average concentrations at 500-520 ppm CO₂ (Mikkelsen *et al.* 2008). Soil water content (SWC) fluctuated with incoming precipitation ranging from c. 25 % down to 5 % in 0-20 cm depth (Fig. 1). In the controls the mean soil water content in 0-60 cm depth gradually decreased from 11.3 % in May, 10.6 % in June to 8.3 % in July, after which it increased to 13.0 % in August due to heavy precipitation, and stayed at this level in autumn, with 11.6 % in September and 13.3% in October. The drought significantly decreased the SWC in July to 6.0% and in August to 9.5 % and decreased in elevated CO₂ in July. Warming decreased SWC in June, July August and September. The interaction between elevated CO₂ and the experimental drought in July (tendency) and August (significant) revealed elevated CO₂ to compensate for the negative effects of drought keeping SWC values at about the same values in all CO₂ treatments (Fig. 2).

Plant water potential

In June the mean xylem plant water potential (PWP) in the controls were about -3.5 bars, decreased down to -5.0 bars in August and then increased to -3.0 bars in September and above -1 bar in October (Fig. 3). The PWP were decreased by drought in July, August and September and by warming in July and September. In July the PWP were synergistically decreased by the combination TD, being lower than in T and D individually.

Leaf gas-exchange

Net photosynthesis (P_n) in the control plots during the growing season ranged from 3.6 ± 0.3 to 10.8 ± 1.4 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ and decreased significantly from May to July after which P_n increased to maximal values in October (Fig. 4). Stomatal conductance (g_s) in the control plots decreased significantly from May through July (all $p < 0.0001$; Fig. 5). The highest g_s were seen in August and October, significantly higher than in September ($p < 0.0001$; Fig. 5). The water use efficiency (WUE) varied from about 2.5 to 5 $\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$, with a seasonal low in August and highest WUE in May and October ($p < 0.0001$; Fig. 6).

Across the season the elevated CO_2 significantly increased P_n (Fig. 4), WUE (Fig. 6), and the photosynthetic nitrogen use efficiency (PNUE, not shown) (all $p < 0.0001$) and there was a clear effect of time for all parameters (all $p < 0.0001$, data not shown).

In May elevated CO_2 increased P_n (Fig. 4) and WUE (Fig. 6) and there was a tendency to increased P_n in response to warming. There were no effects on g_s in May. In June the $[\text{CO}_2]$ increased the P_n and WUE. In July the $[\text{CO}_2]$ increased P_n and WUE. There was a tendency of $T \times D$ interaction on WUE, as WUE were higher in response to TD together compared to T and D alone. In agreement with this, the combination reduced g_s . In August $[\text{CO}_2]$ increased and $[D]$ decreased the P_n . The response to $[T]$ were bi-directional, with a marginal increase of P_n in ambient CO_2 plots, but reduced P_n when combined with elevated CO_2 , as shown by a significant $T \times \text{CO}_2$ interaction. The g_s tended to decrease in $[\text{CO}_2]$ and decreased in $[D]$. The interaction $D \times \text{CO}_2$ on g_s revealed a decrease compared to control and the combined response to be intermediary not differing from neither $[D]$ nor $[\text{CO}_2]$. WUE increased in $[\text{CO}_2]$, decreased slightly in $[D]$ and there was a tendency of $[T]$ to decrease P_n . In September, there were no effects of $[T]$, $[D]$,

[CO₂], or interactions on P_n. The g_s tended to be decreased in [D] and significantly decreased in [CO₂]. The WUE was increased in [CO₂]. In October the P_n and WUE were increased in [CO₂] and there were an interaction between [T] and [CO₂] on P_n and g_s. The T*CO₂ interaction revealed a bidirectional response to [T] as [T] decreased P_n and g_s in ambient plots, but increased these when combined with elevated CO₂.

Leaf and shoot characteristics

The green-to-brown ratio is a measure of current year's growth and vitality of *Calluna*. The ratio was high in May and June, decreased to a medium level in July and was low in August (data not shown). New shoots formed in September leading to high ratio during September through October (not shown). In August the green-to-brown ratio decreased in response to [D] while it tended to increase in September and in October in response to [CO₂]. In response to [T] it increased in August, but tended to decrease in September.

The specific leaf area, SLA, increased in significant steps during each month of measurements from May to August as leaves increased their area, but when the new shoots formed in September the SLA increased only marginally onwards to October (not shown). In June the SLA tended to be higher in [CO₂] and in July this was significant. In July and September the SLA were lower in [D].

The leaf nitrogen content decreased from May to July and was increasing from a new high level in August to marginally higher in September and October (data not shown). Leaf nitrogen decreased in response to [CO₂] in May, June, August, and September.

The leaf carbon content decreased marginally from about 47% to 45 % from May to August and in the new September leaves the leaf carbon content were about 41% and increased to 42 % in October. In response to treatments the changes were relatively small, but [D] decreased the carbon content in August and [CO₂] decreased the carbon content in September (data not shown).

The C/N ratio increased significantly each month from May through July from which it dropped to a significantly lower level in August, with a further slight decrease in September and October (Fig. 6). The [CO₂] increased the C/N ratio in May, June and August.

The leaf $\delta^{13}\text{C}$ was in all months decreased in elevated CO₂ due to the lower $\delta^{13}\text{C}$ signature of the industrial CO₂ gas (c. -28.1 $\delta^{13}\text{C}$ as opposed to -8.0 in ambient air) added to the FACE plots (Fig. 7). Across season the $\delta^{13}\text{C}$ increased in response to [D] and [T]. Responses to [T] were even stronger in elevated CO₂ as shown by the significant interaction [T*CO₂] across season. [T] decreased $\delta^{13}\text{C}$ in June. The T*CO₂ interaction was significant in May and October because [T] and [CO₂] combined led to decreased $\delta^{13}\text{C}$. There was a D*CO₂ interaction effect in September and October because [D] led to an increase in $\delta^{13}\text{C}$ only when combined with [CO₂].

Discussion

Seasonal growth pattern of *Calluna*

Calluna is a slow growing evergreen shrub classed as a stress tolerant competitor (Grime *et al.* 1988) having adaptive traits for dry and nutrient low habitats e.g. xeromorphic leaves, long leaf life span, low nitrogen content, low nutrient loss and likely low

photosynthetic rates (Aerts 1995). In accordance with this overwintering shoots were greening and high photosynthetic rates were observed for *Calluna* in May while P_n decreased during June and July. In this period the C/N ratio increased as nitrogen uptake did not match the carbon input in the leaves, indicating rapid growth. Although shoots elongated the green-to-brown ratio decreased and SLA increased, which can be ascribed to the decrease in SWC during June-July which probably also caused the decreases in P_n and g_s . This growth pattern demonstrates the negative impact of progressive water limitation due to low amount of precipitation during most of the summer and low water retention by the sandy soil. This is supported by previous studies demonstrating soil water availability control on photosynthesis in *Calluna* (Gordon *et al.* 1999, Llorens *et al.* 2004). In August most *Calluna* plants were in flowering and nitrogen was allocated to these and the new shoots which grew from the top of the flowering shoots in the end of the flowering period. This led to the high nitrogen concentration in August, September and October and the parallel decrease in the C/N ratio. During September and October there was a stable C/N ratio, and a marginal increase in the green-to brown ratio and SLA. In autumn the photosynthetic rates were higher and photosynthetic capacity up-regulated as shown by increased V_{cmax} , J_{max} and PNUE (data not shown) probably due to the combination of vital new shoots and increasing water availability. This demonstrates that the evergreen *Calluna* has potential for stimulated carbon assimilation in response to warming mediated growing season increments. Likely this growth potential persist until frost hardening begins and lower temperatures in combination with lower water availability causes winter browning of the shoots (Jackson *et al.* 1999, Watson *et al.* 1966). This short term response is in accordance with the notion that stimulation of

productivity caused by experimental warming is dependent upon aridity (Penuelas *et al.* 2007).

Treatments influence growing season length

Across a range of warming experiments with different approaches and in different ecosystems increases in measures of aboveground productivity have been found (Rustad *et al.* 2001, Arft *et al.* 1999). Across a European gradient the productivity stimulation in response to passive nighttime warming was demonstrated to depend on water availability (Peneuleas *et al.* 2007, Llorens *et al.* 2004). Responses in these studies were argued to result from direct effects via increased photosynthesis at higher temperature, longer growing season or from indirect effects of increased nutrient or water availability (Arft *et al.* 1999, Llorens *et al.* 2004, Peneuleas *et al.* 2007, Rustad *et al.* 2001).

In our study the 33% higher GDD from 1st April to 15th May in warmed plots clearly points to potential for stimulated growth and advanced spring. Indeed the net photosynthesis tended to increase in response to warming, but also in response to elevated CO₂ on the 18th May when the first measurements were conducted. At this time enhanced CO₂ had caused increased leaf C/N ratio and the increased uptake of added CO₂ (with low $\delta^{13}\text{C}$) decreased the $\delta^{13}\text{C}$ when combined with warming, causing the significant T*CO₂ interaction. Hence, spring growth was synergistically stimulated by the combination of warming and elevated CO₂. This suggests the impact of warming in this period to be mediated via shift in phenology which warming have been reported to affect previously (Penuelas *et al.* 2007, Menzel *et al.* 2006, Jensch *et al.* 2008). However, even though stimulated productivity was expected in response to elevated CO₂ (Ainsworth and

Long 2005, Ainsworth and Rogers 2007, Drake *et al.* 1997) it is noteworthy that warming and elevated CO₂ acted synergistically and effects thus could not be deduced from the above single factor expectations. Also in the autumn the T*CO₂ interaction was due to stimulated plant carbon uptake. The temperature was unusual high during September–November 2006 (DMI), and the impact of the experimental passive nighttime warming was probably not as strong as it would have been if a large number of cold nights had occurred. Together with sufficient soil water content these conditions prolonged the growing season leading to the highest growing season net photosynthesis figures occurring in October. The P_n was even further increased in elevated CO₂ plots leading to more growth as the elevated CO₂ also increased the green-to-brown ratio. The warming and elevated CO₂ synergistically increased net photosynthesis, which led to increased growth and decreased $\delta^{13}\text{C}$ in the combined warming and elevated CO₂ plots in October. The synergistically increased carbon uptake in spring and autumn in response to the combination of elevated CO₂ and warming have several implications. The C/N ratio was below 25 and leaf carbon uptake was balanced by nitrogen supply. These conditions clearly increased the carbon sink strength of the vegetation and lengthening of spring and autumn have strong impacts the yearly productivity of this species especially when warming is combined with enhanced CO₂.

Direct effects of the night time warming treatment during day is possible as the effect was still evident 3–5 h after sunrise (Mikkelsen *et al.* 2008). In principle this could provide closer to optimal growth temperature in warmed plots, and increase photosynthetic rates (Sage and Kubien 2007). However, acclimation of respiration to temperature may occur (Atkin and Tjoelker 2003). More likely and in line with other

studies the experimental warming caused indirect effects via altered growing season length and phenology (Harte and Shaw 1995, Wan *et al.* 2005) and increased soil nitrogen mineralization and availability (Peterjohn *et al.* 1994, Rustad *et al.*, 2001). The increased sink strength may also be related to increased carbon cycling in part via increased nighttime leaf respiration (Turnbull *et al.* 2002, 2004) or soil respiration (Piao *et al.* 2007, Schindlbacher *et al.* 2009). Dominance of indirect effects is in line with Shaver *et al.* (2000) providing a conceptual framework for analyzing effects of warming on ecosystem processes, but also adds to the complexity when predictions are to be made.

Water consumption, drought and rewetting affect the photosynthetic performance

The soil water content in 0-60 cm decreased from May to June and on to July and was accompanied by decreased stomatal conductance and reduced transpiration, in accordance with expected association between soil water availability and stomatal conductance (Schulze 1986, Flexas and Medrano 2002). In May to July the net photosynthesis was increased in elevated CO₂ which led to increased water use efficiency as stomatal conductance was unaffected by elevated CO₂. The increased net photosynthesis was the result of increased intercellular CO₂ (data not shown) allowing for higher substrate availability for Rubisco in accordance with previous summaries on effects of elevated CO₂ (e.g. Ainsworth and Long 2005, Ainsworth and Rogers 2007).

The experimental drought decreased the SWC in July and August, but not before August negative effects on net photosynthesis were measured, although in July water relations were clearly negatively affected. This supplements the earlier reported short term acclimation in *Calluna* to experimental drought decreasing the plant water potential

(Jackson *et al.* 1995), light saturated net photosynthesis, transpiration, stomatal conductance, but with little to no effect on WUE (Gordon *et al.* 1999a, 1999b). Further the plant water potential decreased in response to warming and drought in parallel with the SWC, but this did not affect stomatal conductance and transpiration. However, warming and drought decreased synergistically the PWP, stomatal conductance and transpiration, as a consequence of the very low SWC in plots with warming and drought combined, partly due to increased water consumption per leaf area during June-July in TD plots. Notably, the SWC was higher in elevated CO₂ plots compared to ambient plots during summer, clearly suggesting an improved water economy for plants in elevated CO₂. This enables the plants to continue growth when water limitation starts to affect ambient plots. Also the earlier start of the growing season in response to warming leads to increased water consumption causing negative impact on water availability later on, most pronounced in the experimental drought plots.

Generally the photosynthesis stimulation and stomatal conductance decreases are very robust responses in elevated CO₂ (Ainsworth and Long 2005, Ainsworth and Rogers 2007) which results in reduced soil water depletion that mitigates negative effects of drought and sustain net photosynthesis in dry periods (Hungate *et al.* 1997b, Leuzinger and Körner 2007, Morison and Gifford 1984, Robredo *et al.* 2007). Surprisingly, our data did not demonstrate stomatal conductance decreases in elevated CO₂ and the $\delta^{13}\text{C}$ cannot reveal if such decreases potentially occurred between campaigns as the industrial CO₂ dosed in FACE plots differ from the atmospheric signature. Other mechanism may explain the observed soil water savings. In long term changes in the ratio of leaf to sapwood area may occur in *Calluna* (Jackson *et al.* 1999) as in other woody plants

(Margoils *et al.* 1995, Mencuccini and Grace 1995), but the increased SLA and carbon uptake in elevated CO₂ and the decreases in experimental drought as well as the short duration of the manipulations clearly suggest that this did not take place. We speculate that the co-occurring grass species in which major proportion of leaves wilted in response to low water availability (Albert *et al.* 200X) may increase evaporation in ambient conditions to a larger degree than under elevated CO₂, leading to the higher SWC in elevated CO₂. Even if stomatal conductance decreased this does not necessarily translate into reduced transpiration as leaf temperature, wind speed, vegetation roughness and density, vapor pressure deficit, atmospheric temperature, humidity and pressure are factors that may increase the water loss from plant surface and also soil (Amthor 1999, Morgan *et al.* 2004, Field *et al.* 1995, Jacobs and de Bruin 1997).

In August, drought still decreased the SWC, PWP, P_n, g_s, T_r, and WUE, although the drought treatments had ended. In response to elevated CO₂ the net photosynthesis in August was increased as it still benefitted from the higher intercellular CO₂ despite the significantly decreased stomatal conductance which took place although neither SWC nor plant water potential was decreased in response to elevated CO₂. This led to decreased transpiration and led to increased water use efficiency. The photosynthetic capacity regulation was possible as new vital shoots with significantly higher nitrogen content was grown in August. This trade-off became more complex when the elevated CO₂ was combined with drought, as shown by the significant interaction of [D*CO₂] on stomatal conductance and transpiration in August, due to different direction of responses by the drought and elevated CO₂ treatments. The effects on growth accumulated into a decreased green-brown ratio in response to drought, but also an increased C/N ratio in

elevated CO₂. These patterns support the strong negative impact from water limitation on photosynthesis during summer, but also that elevated CO₂ very much counteracts this response. Productivity responses in elevated CO₂ are often modulated by water shortage and precipitation patterns (Körner 2000, Volk *et al.* 2002, Knapp *et al.* 2002, Heisler and Weltzin 2006), particularly in grasslands (Morgan *et al.* 2004, Körner 2006, Robredo *et al.* 2007).

The significant T*CO₂ interaction on photosynthesis in August was due to a negative impact of warming and a positive impact of elevated CO₂ which were not to additive. The decrease in photosynthesis in plots where warming and elevated CO₂ were combined was not paralleled by effects on stomatal conductance or transpiration, but via down-regulation of the photosynthetic capacity via decreased V_{cmax} in [T*CO₂] in August (data not shown). The decreased ability to keep photosynthesis high in the combined warming and elevated CO₂ plots probably was due to higher biomass accumulated previously and resulting higher water consumption per ground area. As an evergreen dwarf shrub, *Calluna* protects its investments in woody tissues by down regulating photosynthesis when facing water shortage. Such response pattern highlight a directly counteracting mechanism for the beneficial effect soil water savings if water consumption increased with biomass.

Even after pronounced precipitation in late August and early September the soil water content were still lower in warmed and in drought plots although drought treatment had ended, which reduced the plant water potential here, and tended to decrease stomatal conductance and transpiration in drought plots. Although there were no effects on net photosynthesis the SLA in September was decreased in response to the former drought.

In September the elevated CO₂ decreased stomatal conductance and transpiration and increased the water use efficiency and the green-to-brown ratio increased indicating some stimulation of growth in elevated CO₂ plots. Water shortage was evident when elevated CO₂ was combined with drought as shown by decreasing the less negative $\delta^{13}\text{C}$ in September and in October in drought plots also exposed to CO₂. After the rewetting in October there were no treatment effects on the plant water potential and stomatal conductance were high. This demonstrates that the negative effects of the experimental drought extend beyond drought period itself, but also that *Calluna* as an evergreen dwarf shrub with xeromorphic traits (Aerts 1995, Grime *et al.* 1988) is able to tolerate this.

Conclusion

The earlier start of the growing season in response to warming led to increased water consumption, negative impact on water availability and decreased net photosynthesis and growth. The water availability progressively decreased during the first part of the growing season, why negative effects on net photosynthesis and water economy was first seen in the drought plots. Elevated CO₂ counteracted this until August, after which the drought and elevated CO₂ in combination caused earlier senescence. Plants increased photosynthesis and growth via earlier season start and prolonged autumn in plots where warming and elevated CO₂ were combined. Ecosystem processes and pools take time to acclimate and respond to the stepwise change by which we applied the experimental changes in atmospheric CO₂, temperature and drought. However, understanding of the temporal response patterns and the underlying patterns are fundamental, although initial responses may differ from what may be observed on a longer term basis. Our experiment

clearly demonstrates that the concerted impact of changes in temperature, precipitation and atmospheric CO₂ affect net photosynthesis, have a different strength and impact during season and also interact in a way that could not be deduced from single factor experimentation. In the combined treatment of warming, elevated CO₂ and drought, which best represents the predicted future climate the productivity was increased in early and late growing season were extended. However, this leads to increased water consumption decreasing productivity during summer with low water availability. Whether these results also reflect longer term responses to treatments in combination with the yearly climatic variation is, however, not predictable.

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References

- Aerts R. 1995.** The advantages of being evergreen. *Tree* **10**: 402-406.
- Ainsworth EA, Long S. 2005.** What have we learned from 15 years of Free-Air CO₂ Enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New Phytologist* **165**: 351-372.
- Ainsworth EA, Rogers A. 2007.** The response of photosynthesis and stomatal conductance to rising [CO₂]: mechanisms and environmental interactions. *Plant, Cell and Environment* **30**: 258-270.
- Amthor JS. 1999.** Increasing atmospheric CO₂, water use, and water stress: scaling up from the plant to the landscape. *In*: Carbon Dioxide and Environmental Stress (eds Lou Y., Mooney H.A.) pp 33-59. Academic press, San Diego.
- Albert KR, Kongstad J, Schmidt IK, Ro-Poulsen H, Mikkelsen TN, Michelsen A, Beier C. 200X.** Growth and eco-physiological characteristics of *Calluna vulgaris* and *Deschampsia flexuosa* to natural drought and warm autumn in a temperate heath. *In prepp.*
- Arft AM, Walker MD, Gurevitch J, Alatalo JM, Bret-Harte MS, Dale M, Diemer M, Gugerli F, Henry GHR, Jones MH, Hollister RD, Jónsdóttir IS, Laine K, Lévesque E, Marion GM, Molau U, Mølgaard P, Nordenhäll U, Raszhivin V, Robinson CH, Starr G, Stenström A, Stenström M, Totland Ø, Turner PL, Walker LJ, Webber PJ, Welker JM, Wookey PA. 1999.** Responses of tundra plants to experimental warming: Meta-analysis of the International Tundra Experiment. *Ecological Monographs* **69**: 491-511.
- Atkin OK, Tjoelker MG. 2003.** Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends in Plant Science* **8**: 343-351.
- Beier C, Emmett B, Gundersen P, Tietema A, Penuelas J, Estiarte M, Gordon C, Gorissen A, Llorens L, Roda F, Williams D. 2004.** Novel approaches to study climate change effects on terrestrial ecosystems in the field: Drought and passive nighttime warming. *Ecosystems* **7**: 583-597.
- Chaves MM. 1991.** Effects of water deficits on carbon assimilation. *Journal of Experimental Botany* **42**: 1-16.
- Ciais P, Reichstein M, Viovy N, Granier A, Ogée J, Allard V, Aubinet M, Buchmann N, Bernhofer Chr, Carrara A, Chevallier F, De Noblet N, Friend A D, Friedlingstein P, Grünwald T, Heinesch B, Keronen P, Knohl A, Krinner G, Loustau D, Manca G, Matteucci G, Miglietta F, Ourcival JM, Papale D, Pilegaard K, Rambal S, Seufert G, Soussana JF, Sanz MJ, Schulze ED, Vesala T, Valentini R.**

2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature* **437**: 529-533.

Classen AT, Langley JA. 2005. Data–model integration is not magic. *New Phytologist* **166**: 367 – 370.

Cleland EE, Chiariello NR, Loarie SR, Mooney HA, Field CB. 2006. Diverse responses of phenology to global changes in a grassland ecosystem. *Philosophical Transactions of the National academy of Science* **103**:13740-13744.

Curtis PS, Wang X. 1998. A meta analysis of elevated CO₂-effects on woody plant mass, form and physiology. *Oecologia* **113**: 299-313.

DMI.dk. Danish Meteorological Institute homepage:
http://www.dmi.dk/dmi/index/danmark/meteorologiske_ekstremer_i_danmark.htm

Drake B, Gonzalez-Meler M, Long S. 1997. More efficient plants: a consequence of rising atmospheric CO₂? *Annual Review of Plant Physiology and Plant Molecular Biology* **48**: 609-639.

Dubois J-J B, Fiscus EL, Booker FL, Flowers MD, Reid CD. 2007. Optimizing the statistical estimation of the parameters of the Farquhar-von Caemmerer-Berry model of photosynthesis. *New Phytologist* **176**, 402-414.

Farquhar GD, von Caemmerer S, Berry JA 1980. A biochemical model of photosynthetic CO₂ carbon dioxide assimilation in leaves of C₃ carbon pathway species. *Planta* **149**: 78-90.

Field CB, Jackson RB, Mooney HA. 1995. Stomatal responses to increased CO₂: implications from the plant to the global scale. *Plant, Cell and Environment* **18**: 1214 - 1225.

Flexas J, Medrano H. 2002. Drought-inhibition of Photosynthesis in C₃ plants: Stomatal and Non-stomatal limitations revisited. *Annals of Botany* **89**: 183-189.

Foot JP, Caporn SJM, Lee JA, Ashenden TW. 1996. The effect of long term ozone fumigation on the growth, physiology and frost sensitivity of *Calluna vulgaris*. *New Phytologist* **133**: 503-511.

Gordon C, Woodin SJ, Mullins CE, Alexander IJ. 1999a. Effects of environmental change, including drought, on water use by competing *Calluna vulgaris* (heather) and *Pteridium aquilinum* (bracken). *Functional Ecology* **13**: 96-106.

Gordon C, Woodin SJ, Alexander IJ, Mullins CE. 1999b. Effects of increased temperature, drought and nitrogen supply on two upland perennials of contrasting

functional type: *Calluna vulgaris* and *Pteridium aquilinum*. *New Phytologist* **142**: 243–258.

Grime JP, Hodgson JG, Hunt R. 1988. Comparative plant ecology: a functional approach to common British species. London, United Kingdom, Unwin Hyman.

Harte J, Shaw R. 1995. Shifting dominance within a montane vegetation community: Results of a climate warming experiment. *Science* **267**: 876-880.

Heimann M, Reichstein M. 2008. Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature* **451**: 289-292.

Henry HAL, Cleland EE, Field CB, Vitousek PM. 2005. Interactive effects of elevated CO₂, N deposition and climate change on plant litter quality in a California annual grassland. *Oecologia* **142**: 465-473.

Heisler JA, Weltzin JF. 2006. Variability matters: towards a perspective on the influence of precipitation on terrestrial ecosystems. *New Phytologist* **172**: 189-192.

Hungate BA, Holland EA, Jackson RB, Chapin III FS, Mooney HA, Field CB 1997a. The fate of carbon in grasslands under carbon dioxide enrichment. *Nature* **388**: 576-579.

Hungate BA, Chapin FS II, Zhong H, Holland EA, Field CB. 1997b. Stimulation of grassland nitrogen cycling under carbon dioxide enrichment. *Oecologia* **109**: 149-153.

IPCC. 2001. Climatic change 2001: The Scientific Basis. Intergovernmental Panel on Climate Change, New York, Cambridge University Press.

IPCC. 2007. Climatic change 2007: The Physical Science Basis. Intergovernmental Panel on Climate Change, Geneva.

Jacobs CMJ, de Bruin HAR. 1997. Predicting regional transpiration at elevated atmospheric CO₂: influence of the PBL-vegetation interaction. *Journal of Applied Meteorology* **36**: 1663-1675.

Jackson GE, Irvine J, Grace J. 1999. Xylem acoustic emissions and water relations of *Calluna vulgaris* L. at two climatological regions of Britain. *Plant Ecology* **140**: 3-14.

Jentsch A, Kreylling J, Boettcher-Treschow J, Beierkuhnlein C. 2008. Beyond gradual warming: extreme weather events alter flower phenology of European grassland and heath species. *Global Change Biology* **14**: 1-13.

Knapp KA, Fay PA, Blair JM, Collins SL, Smidth MD, Carlistre JD, Harper CW, Danner BT, Lett MS, McCarron JK. 2002. Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. *Science* **298**: 2202-2205.

Körner C, Asshoff R, Bignucolo O, Hättenschwiler S, Keel SG, Peláez-Riedl S, Pepin S, Siegwolf RTW, Zotz G. 2005. Carbon Flux and Growth in Mature Deciduous Forest Trees Exposed to Elevated CO₂. *Science* **309**: 1360 – 1362.

Körner C. 2000. Biosphere responses to CO₂ enrichment. *Ecological Applications* **10**:1590–1619.

Körner C. 2003. Carbon limitation in trees. *Journal of Ecology* **91**: 4-17.

Körner C. 2006. Plant CO₂ responses: An issue of definition, time and resource supply. *New Phytologist* **172**: 393-411.

Leuzinger S, Körner C. 2007. Water savings in mature deciduous forest trees under elevated CO₂. *Global Change Biology* **13**: 2498–2508.

Llorens L, Penuelas J, Beier C, Emmett BA, Estiarte M, Tietema A. 2004. Effects of an experimental increase of temperature and drought in the photosynthetic performance of two ericaceous shrub species along a north–south European gradient. *Ecosystems* **7**: 613–624.

Luo Y, Gerten D, Maire GL, Parton WJ, Weng E, Zhou X, Keough C, Beier C, Ciais P, Cramer W, Dukes JS, Emmett B, Hanson PJ, Knapp A, Linder S, Nepstad D, Rustad L. 2008. Modelled interactive effects of precipitation, temperature and [CO₂] on ecosystem carbon and water dynamics in different climatic zones. *Global Change Biology* **14**: 1986-1999.

Lou Y. 2007. Terrestrial carbon cycle feedback to climate warming. *Annual Review of Ecology, Evolution and Systematics* **38**: 683-712.

Lou Y, Su B, Currie WS, Dukes JS, Finzi A, Hartwig U, Hungate B, McMurtrie RE, Oreon R, Parton WJ, Pataki DE, Shaw MR, Zak DR, Field CB. 2004. Progressive Nitrogen Limitation of Ecosystem Responses to Rising Atmospheric Carbon Dioxide. *Bioscience* **54**: 731-739.

Margoils H, Oren R, Whitehead D, Kaufman M. 1995. Leaf area dynamics of conifer forests. In: Smith W.K., Hinckley T.M. (eds.) *Ecophysiology of coniferous forests*. Academic Press. San Diego, London. Pp 181-223.

Mencuccini M, Grace J. 1995. Climate influence the leaf area-sapwood area relationship in Scots pine (*Pinus sylvestris* L.) *Tree Physiology* **15**: 1-10.

Menge DNL, Field CB. 2007. Simulated global changes alter phosphorus demand in annual grassland. *Global Change Biology* **13**: 2582-2591.

Menzel A, Fabian P. 1999. Growing season extended in Europe. *Nature* **397**: 659.

Menzel A, Sparks TH, Estrella N, Koch E, Aasa A, Ahas R, Alm-Kübler K, Bissolli P, Braslavská O, Briede A, Chielewski FM, Crepensek Z, Curnel Y, Dahl Å, Defila C, Donnelly A, Filella Y, Jatzczak K, Måge F, Mestre A, Nordli Ø, Penuelas J, Pirinen P, Remosova V, Scheifinger H, Striz M, Susnik A, van Vliet AJH, Wielgolaski F-E, Zach S, Züst A. 2006. European phenological response to climate change matches the warming pattern. *Global Change Biology* **12**: 1969-1976.

Mikkelsen TN, Beier C, Jonasson S, Holmstrup M, Schmidt IK, Ambus P, Pilegaard K, Michelsen A, Albert KR, Andresen LC, Arndal MF, Bruun N, Christensen S, Danbæk S, Gundersen P, Jørgensen P, Kongstad J, Maraldo K, Primé A, Riis-Nielsen T, Ro-Poulsen H, Stevnbak K, Selsted MB, Sørensen P, Larsen KS, Carter MS, Martinussen T, Miglietta F, Sverdrup H. 2008. Experimental design of multifactor climate change experiments with elevated CO₂, warming and drought – the CLIMATE project. *Functional Ecology* **22**: 185-195.

Moore BD, Cheng S-H, Sims D, Seemann JR. 1999. The biochemical and molecular basis for photosynthetic acclimation to elevated atmospheric CO₂. *Plant Cell and Environment* **22**: 567-582.

Morison JIL, Gifford RM. 1984. Plant growth and water use with limited water supply in high CO₂ concentrations. II. Plant dry weight, partitioning and water use efficiency. *Australian Journal of Plant Physiology* **11**: 375–384.

Morgan JA, Pataki DE, Körner C, Clark H, Del Grosso SJ, Grünzweig JM, Knapp AK, Mosier AR, Newton PCD, Niklaus PA, Nippert JB, Nowak RS, Parton WJ, Polley HW, Shaw MR. 2004. Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO₂. *Oecologia* **140**: 11–25.

Niklaus PA, Spinnler D, Körner C. 1998. Soil moisture dynamics of calcareous grassland under elevated CO₂. *Oecologia* **117**: 201–208

Norby RJ, Lou Y. 2004. Evaluating ecosystem responses to rising atmospheric CO₂ and global warming in a multi-factor world. *New Phytologist* **162**: 281-294.

Norby RJ, Rustad LE, Dukes JS, Ojima DS, Parton WJ, Del Grosso SJ, McMurtrie RE, Pepper DA. 2007. Ecosystem responses to warming and interacting global change factors. *In: Terrestrial Ecosystems in a Changing World* (Canadell J, Pataki D, Pitelka L eds), pp. 45-58, Springer, New York.

Oren R, Ellsworth DS, Johnsen KH, Phillips N, Ewers BE, Maier C, Schäfer KVR, McCarthy H, Hendry G, McNulty SG, Katul GC. 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO₂ enriched atmosphere. *Nature* **411**: 469-472.

Penuelas J, Filella I. 2001. Responses to a warming world. *Science* **294**: 793-795.

Peñuelas J, Prieto P, Beier C, Cesaraccio C, de Angelis P, de Dato G, Emmett BA, Estiarte M, Garadnai J, Gorissen A, Lang EK, Kröel-Dulay G, Llorens L, Pellizzaro G, Riis-Nielsen T, Schmidt IK, Sirca C, Sowerby A, Spano D, Tietma A. 2007. Response of plant species richness and primary productivity in shrublands along a north-south gradient in Europe to seven years of experimental warming and drought: reductions in primary productivity in the heat and drought year of 2003. *Global Change Biology* **13**: 2563-2581.

Peterjohn WT, Adams MB, Gilliam FS. 1994. Symptoms of nitrogen saturation in two central Appalachian hardwood forest ecosystems. *Biogeochemistry* **35**: 507-522.

Piao S, Friedlingstein P, Ciais P, Viovy N, Demarty J. 2007. Growing season extension and its impact on terrestrial carbon cycle in the Northern Hemisphere over the past 2 decades, *Global Biogeochemical Cycles* **21**: GB3018. Doi:10.1029/2006GB002888.

Reich PB, Hobbie SE, Lee T, Ellsworth DS, West JB, Tilman D, Knops JMH, Naeem S, Trost J. 2006. Nitrogen limitation constrains sustainability of ecosystem response to CO₂. *Nature* **440**: 922-925.

Robredo A, Pérez-López U, Sainz de la Maza H, González-Moro B, Lacuesta M, Mena-Petite A, Munoz-Rueda A. 2007. Elevated CO₂ alleviates the impact of drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis. *Environmental and Experimental Botany* **59**: 252-263.

Rustad L, Campbell J, Marion G, Norby R, Mitchell M, Hartley A, Cornelissen J, Gurevitch J, GCTE-NEWS. 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* **126**: 543–562.

Rustad LE. 2006. From transient to steady state response of ecosystems to atmospheric CO₂-enrichment and global climate change: Conceptual challenges and need for an integrated research. *Plant Ecology* **182**: 43-62.

Rustad LE. 2008. The response of terrestrial ecosystems to global climate change: Towards an integrated approach. *Science of the total environment* **404**: 222-235.

Schindlbacher A, Zechmeister-Boltenstern S, Jandl R. 2009. Carbon losses due to soil warming: Do autotrophic and heterotrophic soil respiration respond equally? *Global Change Biology*. DOI 10.1111/j.1365-2486.2008.01757.x

Schulze E-D. 1986. Carbon dioxide and water vapor exchange in response to drought in the atmosphere and soil. *Annual review of Plant Physiology* **37**: 247-274.

Shaver GR, Canadell J, Chapin III FS, Gurevitch J, Harte J, Henry G, Ineson P, Jonasson S, Melillo J, Pitelka L, Rustad L. 2000. Global warming and terrestrial ecosystems: a conceptual framework for analysis. *Bioscience* **50**: 871-882.

Shaw MR, Zavaleta ES, Chiariello NR, Cleland EE, Mooney HA, Field CB. 2002. Grassland responses to global environmental changes suppressed by elevated CO₂. *Science* **298**: 1987-1990.

Sage RF, Kubien DS. 2007. The temperature response of C3 and C4 photosynthesis *Plant, Cell & Environment* **30**: 1086-1106.

Turnbull MH, Tissue DT, Murthy R, Wang X, Sparrow AD, Griffin KL. 2004. Nocturnal warming increases photosynthesis at elevated CO₂ partial pressure in *Populus deltoids*. *New Phytologist* **161**: 819-826.

Turnbull MH, Murthy R, Griffin KL. 2002. The relative impacts of daytime and night time warming on photosynthetic capacity in *Populus deltoides*. *Plant, Cell and Environment* **25**: 1729-1737.

Volk M, Niklaus PA, Körner C. 2000. Soil moisture effects determine CO₂ responses of grassland species *Oecologia* **125**:380–388.

Walther G-R, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, Fromentin J-M, Hoegh-Guldberg O, Bairlein F. 2002. Ecological responses to recent climate change. *Nature* **416**: 389-395.

Wan S, Hui D, Wallace L, Lou Y. 2005. Direct and indirect effects of experimental warming on ecosystem carbon processes in a tall grass prairie. *Global Biochemical Cycles* **19**: GB2014. doi:10.1029/2004GB002315.

Wan S, Luo Y, Wallace LL. 2002. Changes in microclimate induced by experimental warming and clipping in tallgrass prairie. *Global Change Biology* **8**: 754–768.

White MA, Running SW, Thornton PE. 1999. The impact of growing-season length variability on carbon assimilation and evapotranspiration over 88 years in the eastern US deciduous forest. *International Journal of Biometeorology* **42**:139–145.

Yordanov I, Velikova V, Tsonev T. 2000. Plant responses to drought, acclimation, and stress tolerance. *Photosynthetica* **38**: 171-186.

Figure 1: Seasonal variation in climate parameters.

Shown are in (upper) the daily averages of soil water content in control plots in 0-20 cm and 0-60 cm depth and daily accumulated precipitation, (middle) daily averages of air temperature in 2m height and (lower) photosynthetically active radiation (PAR).

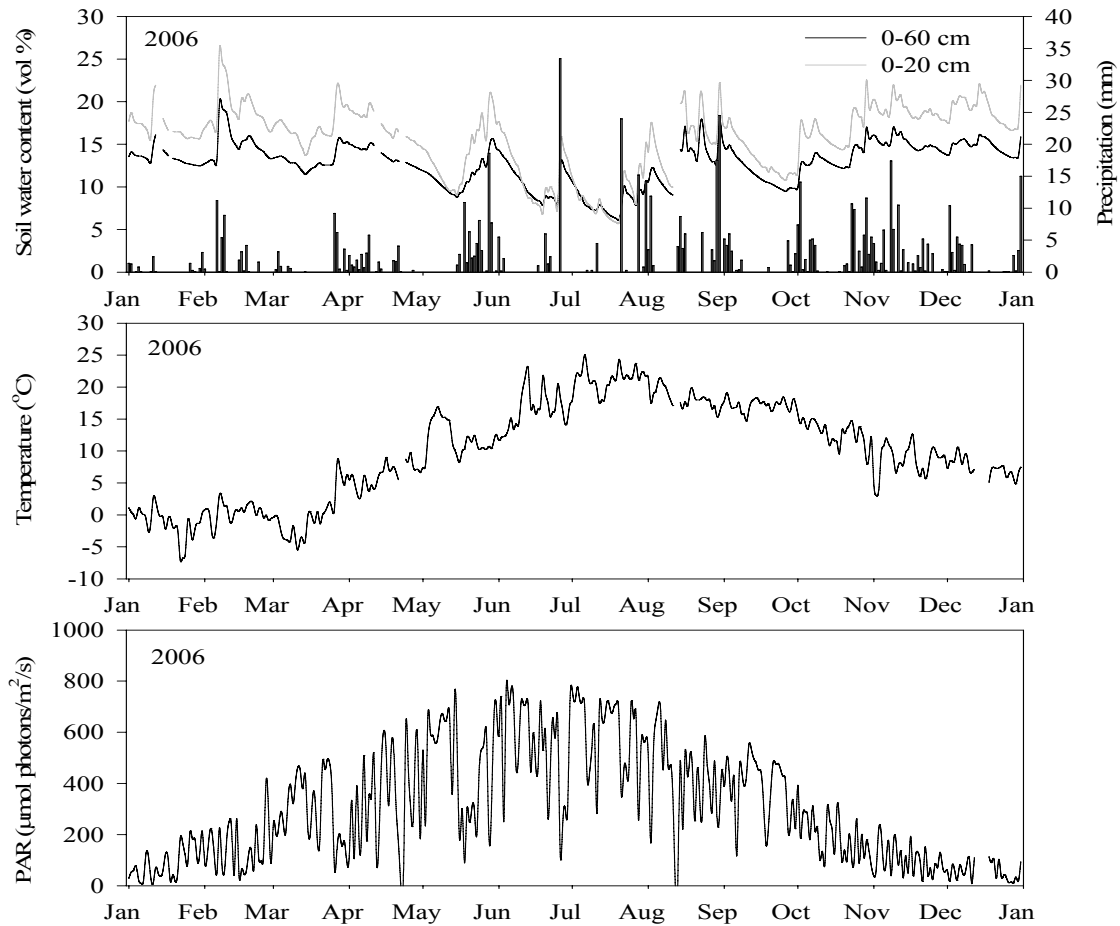


Figure 2: Soil water content in 0-60 cm depth.

Shown is monthly mean \pm standard deviation. Treatments are (A) un-manipulated control, (T) passive nighttime warming, (D) experimental drought, active in July- early August, (TD) warming and drought combined, (CO₂) elevated CO₂, (TCO₂) warming and elevated CO₂ combined, (DCO₂) drought and elevated CO₂ combined, (TDCO₂) warming, drought and elevated CO₂ combined. Statistically significant factors and interactions are shown with treatment letters and levels of significance with *** if $p < 0.0001$, ** if $p < 0.001$, * if $p < 0.05$ and † if $p < 0.10$.

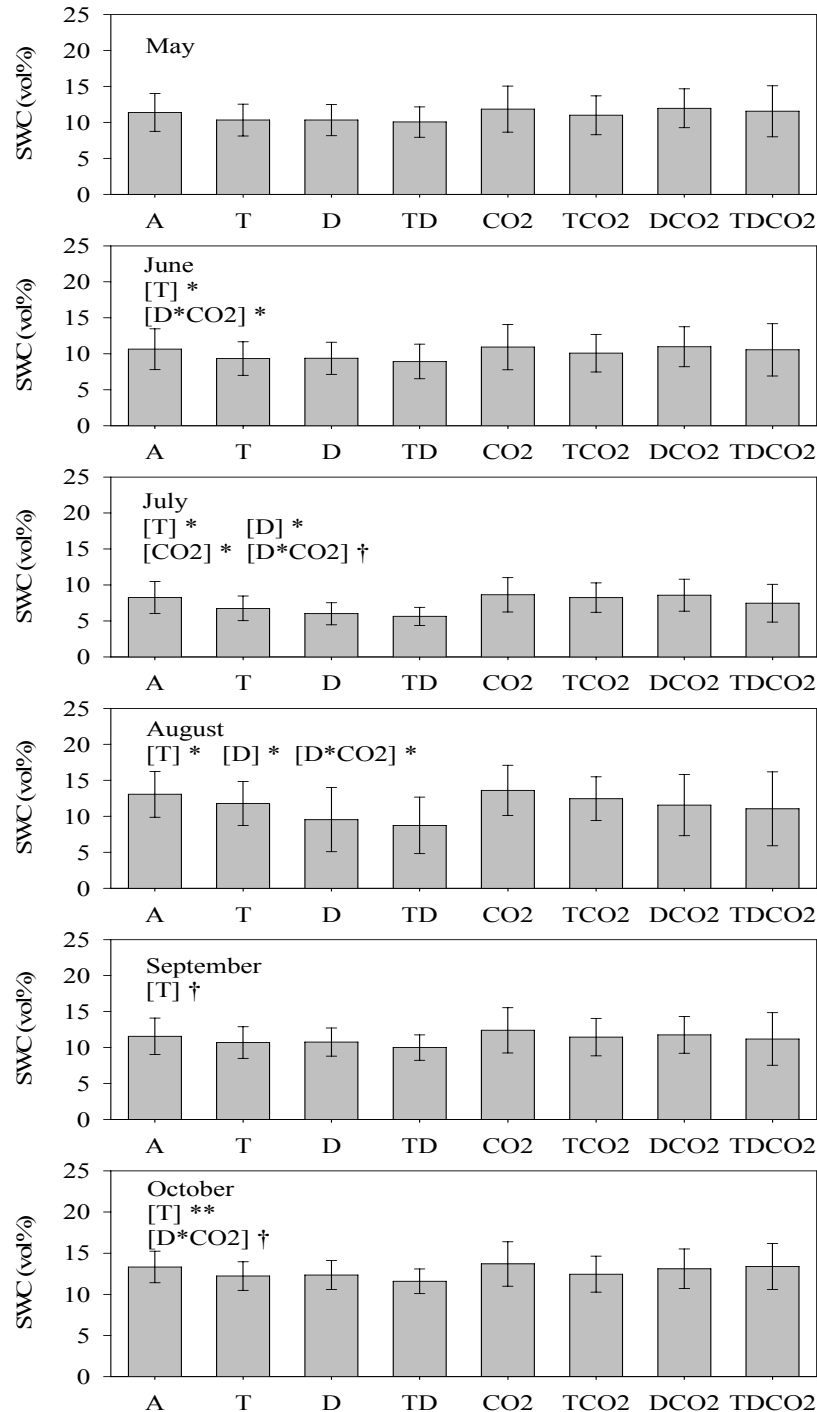


Figure 3: Plant water potential.
Shown are the mean \pm standard errors. Treatments abbreviations and statistics as in figure 2.

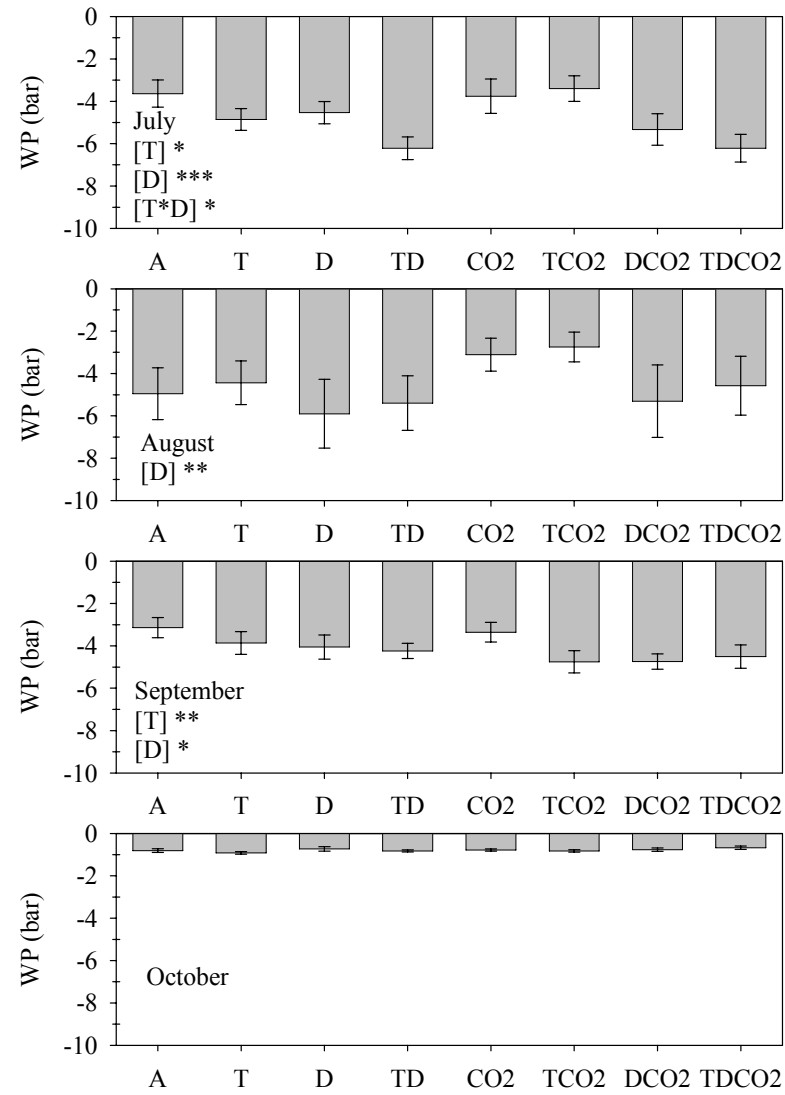


Figure 4: Light saturated net photosynthesis of *Calluna*.

Shown are the mean \pm standard errors. Treatments abbreviations and statistics as in figure 2.

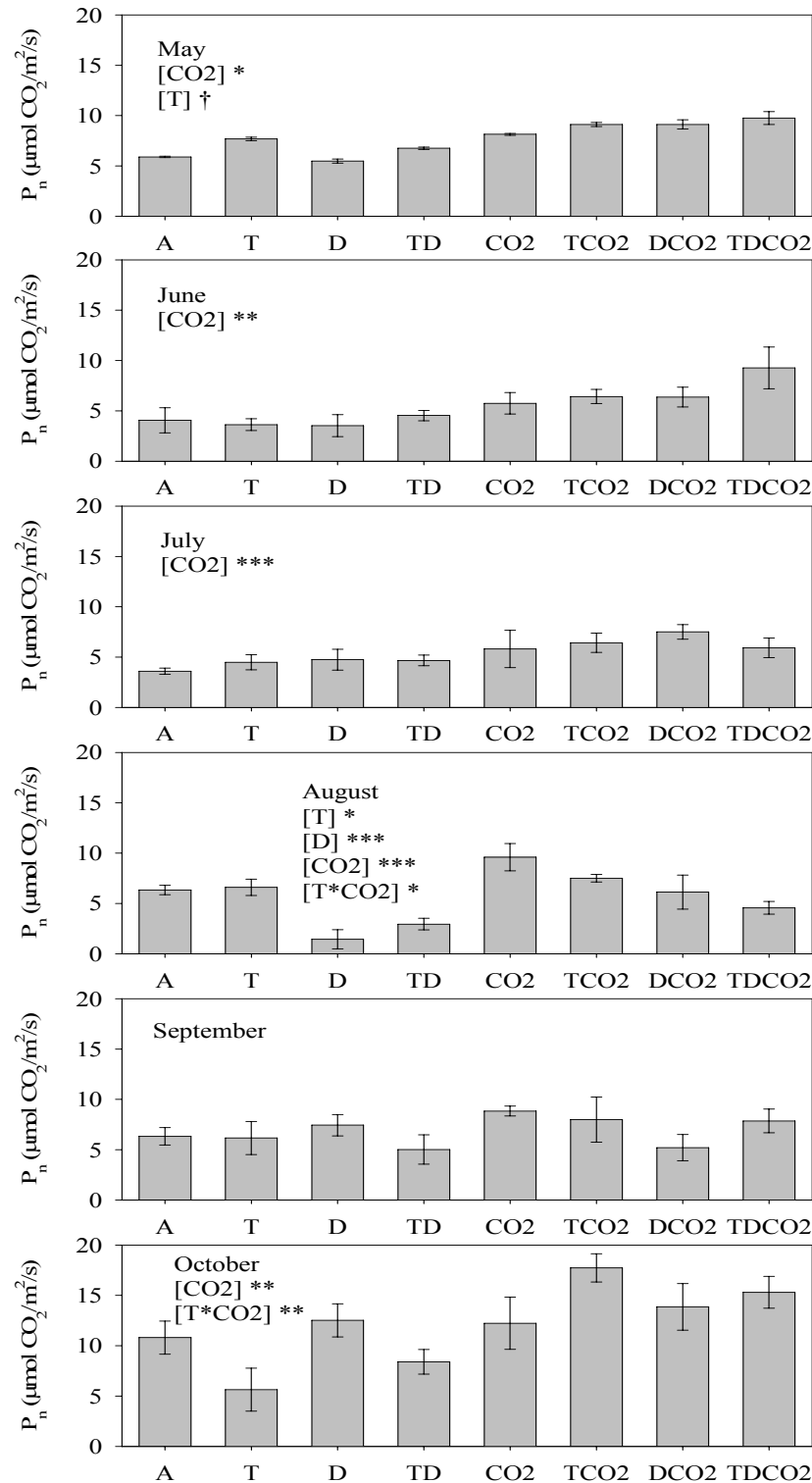


Figure 5: Stomatal conductance of *Calluna*.

Shown are the mean \pm standard errors. Treatments abbreviations and statistics as in figure 2.

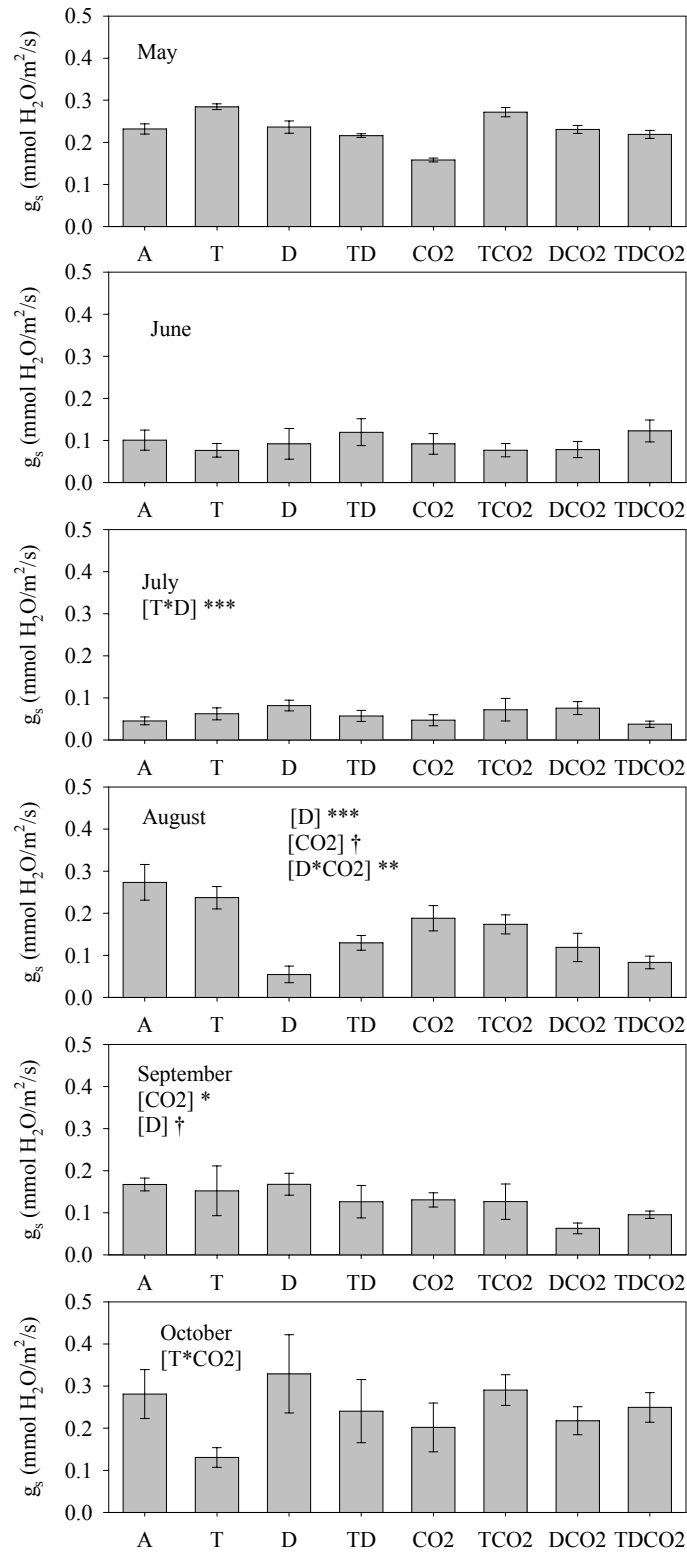


Figure 6: Water use efficiency of *Calluna*.

Shown are the mean \pm standard errors. Treatments abbreviations and statistics as in figure 2.

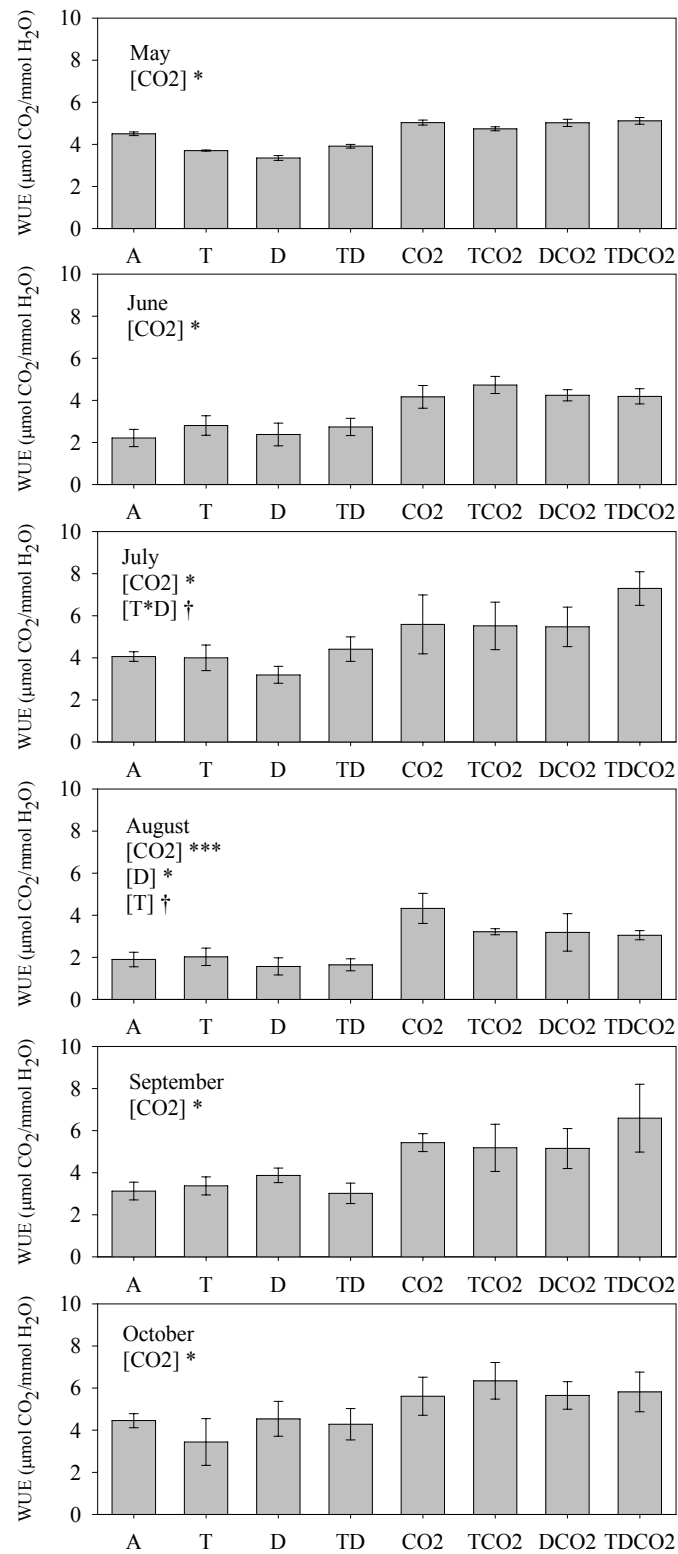


Figure 7: Leaf carbon to nitrogen ratio of *Calluna*.

Shown are the mean \pm standard errors. Treatments abbreviations and statistics as in figure 2.

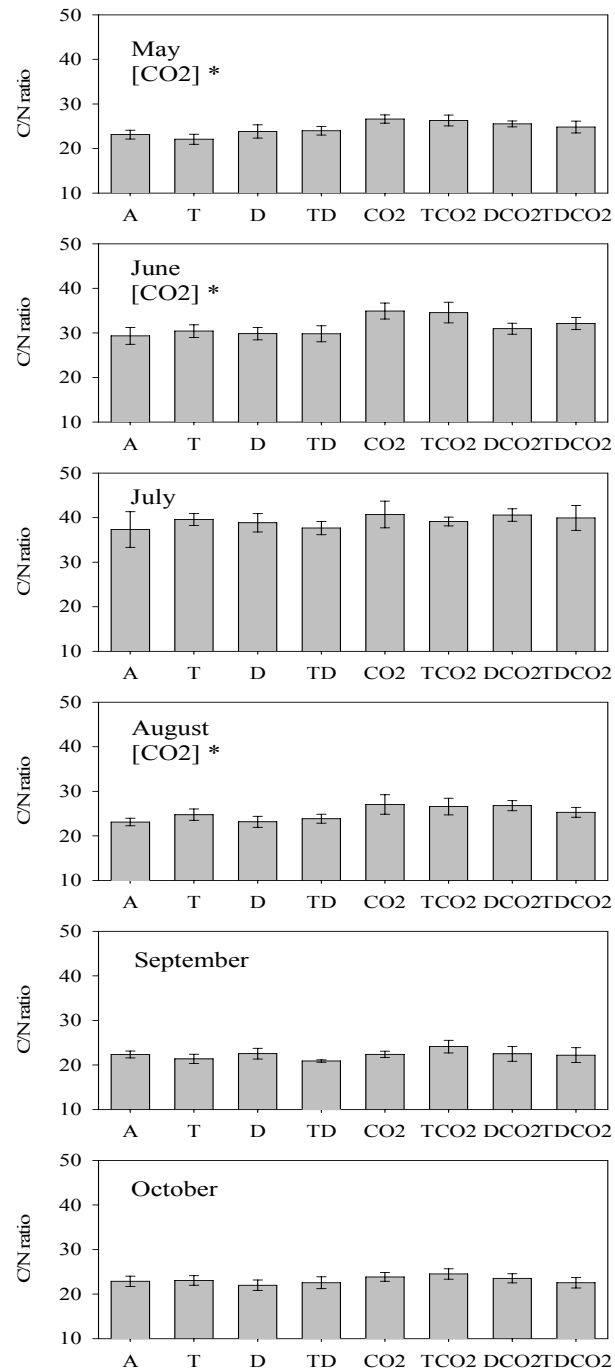
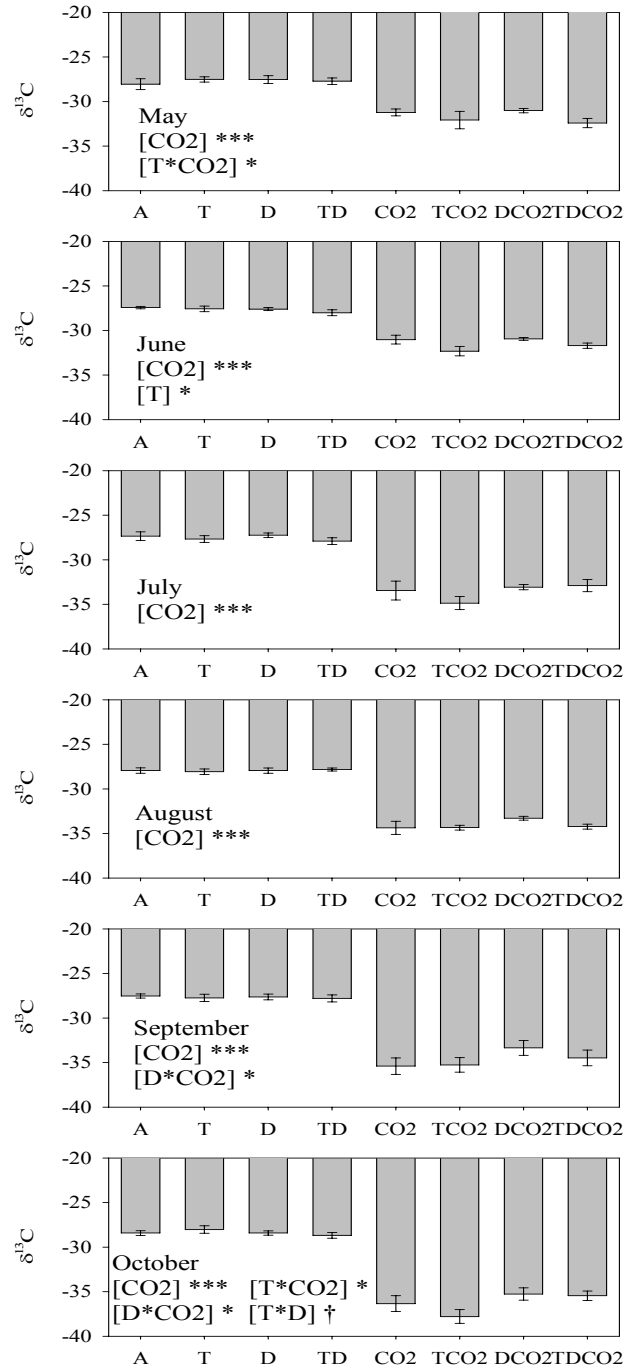


Figure 8: Natural abundance of leaf $\delta^{13}\text{C}$ in *Calluna*.

Shown are the mean \pm standard errors. Treatments abbreviations and statistics as in figure 2. Note that the industrial CO_2 gas dosed in the FACE plots had a lower $\delta^{13}\text{C}$ signature (c. -28.1 $\delta^{13}\text{C}$ as opposed to -8.0 in ambient air) causing the significantly lower $\delta^{13}\text{C}$ values in leaves in elevated CO_2 treatment.



Paper IV

**Photosynthetic performance differences in response to elevated CO₂,
warming and drought: Comparing an opportunist grass to an evergreen
dwarf shrub in a temperate heath ecosystem.**

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Manuscript

Photosynthetic performance differences in response to elevated CO₂, warming and drought: Comparing an opportunist grass to an evergreen dwarf shrub in a temperate heath ecosystem.

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List of abbreviations:

Maximal velocity of RuBP regeneration (J_{\max}), Maximal velocity of Rubisco carboxylation (V_{\max}), Light and CO₂ saturated net photosynthesis (P_{\max}), Light saturated net photosynthesis at ambient CO₂ (P_n), Stomatal conductance (g_s), Leaf nitrogen concentration (N), Soil water content (SWC), Passive night time warming (T), experimental drought (D), experimental elevated CO₂ (CO₂)

Keywords:

CLIMAITE, multi factor experiment, net photosynthesis, heath ecosystem, *Deschampsia flexuosa*, FACE, drought, warming, P_{\max} , J_{\max} , V_{\max} , grassland

Abstract

Increased atmospheric CO₂, warming and changed precipitation patterns impact ecosystem structure and processes. The presence of non-additive effects when these factors are combined is adding complexity and challenges to our current understanding. We therefore investigated the impact of elevated CO₂ (FACE), periodic drought (automatized water excluding curtains) and warming (nighttime IR-reflective curtains) on photosynthesis and leaf characteristics on the evergreen dwarf shrub *Calluna vulgaris* and the grass *Deschampsia flexuosa* in a multi-factorial experiment, in a temperate heath ecosystem. We investigated the photosynthetic performance responses towards these manipulations by characterizing the photosynthetic capacity and carbon sink strength during growing season. Warming improved photosynthetic capacity in both growth forms during spring and early summer. Rigid photosynthetic capacity was demonstrated in the dwarf shrub. Adjustments were almost only observed when elevated CO₂ was combined with severe drought. In response to drought alone the dwarf shrub maintained metabolism and photosynthetic capacity. Variable photosynthetic capacity was demonstrated in the grass. In response to drought it wilted and the remaining leaves had low photosynthetic capacity. In elevated CO₂ increased photosynthetic capacity was observed when soil water availability allowed for luxurious water consumption but this completely reversed when SWC were unfavorable. Rewetting caused sprouting of new grass leaves with high nitrogen content and photosynthetic capacity. The results clearly showed that water relations clearly modulated the photosynthetic performance responses to elevated CO₂ and warming and have a strong impact on N-availability. The responses of the two species were much influenced by their different growth strategies.

Introduction

Environmental changes caused by increased emissions of greenhouse gasses have been predicted to influence the stability of ecosystems worldwide (IPCC 2001). For terrestrial plants and ecosystems increases in atmospheric CO₂ concentration and air temperature, and changes in precipitation patterns are expected to have strong impacts on carbon balance. Regional climate modelling show enhanced temperature and a decrease in precipitation in Denmark during summer resulting in longer drought periods and occasional incidences of high amounts of precipitation (Christensen and Christensen 2003). Effects of changes in the environment such as elevated CO₂, temperature and precipitation on plants and ecosystems have primarily been investigated as effects of a single factor or two factors in combination (e.g. Beier *et al.* 2004, for overview see Rustad 2008). Since all environmental changes will occur concurrently and all factors could interact it is important to study the effects of the factors alone and in combination in order to predict the outcome of multiple changes for plants and ecosystems processes in a changed climate.

Warming generally increases primary productivity (Dukes *et al.* 2005, Penuelas *et al.* 2007, Rustad *et al.*, 2001, Shaw *et al.*, 2002), but the magnitude may strongly depend on aridity (Penuelas *et al.* 2007). Warming also increases the length of the growing season (Cleeland *et al.* 2006, Menzel and Fabian 1999, Walter *et al.* 2002) and changes plant phenology (Harte and Shaw 1995, Menzel *et al.* 2006, Wan *et al.* 2005), increases soil nitrogen mineralization and availability (Rustad *et al.* 2001), reduces soil water content (Morgan *et al.* 2004, Volk *et al.* 2000, Wan *et al.* 2005) and affects species composition and community structure (Shaver *et al.* 2000, Wan *et al.*, 2005). The indirect

effects on photosynthesis and productivity have been argued to be more important than direct effects (Körner 2000, Lou 2007, Morgan *et al.*, 2004, Shaver *et al.* 2000).

Elevated CO₂ predominantly decreases stomatal conductance, stimulates the light saturated net photosynthesis and increases plant water use efficiency (Ainsworth and Rogers 2007, Ainsworth and Long 2005, Curtis 1996, Curtis and Wang 1998). Elevated CO₂ often down-regulate the photosynthetic capacity via the maximal carboxylation velocity of Rubisco, V_{cmax} , and to less degree also the maximal rate of RubB regeneration, J_{max} , decreases (Moore *et al.* 1999, Drake *et al.* 1997, Ainsworth and Rogers 2007). This is in part due to buildup of carbon compounds and other factors limiting export of carbon compounds produced in the Calvin Cycle, and in part due to limitations in the nitrogen supply (Ainsworth and Rogers 2007, Drake *et al.* 1997). Plant carbon sink strength may further be determined by carbon demand for e.g. storage, growth, metabolism or export and therefore is dependent on both the residence time in the carbon pools and the flux rates between them (Körner 2006). Hence, sink strength may partly be sustained via increased carbon cycling as reported from grassland (Hungate *et al.* 1997) and forest (Körner 2005) in response to elevated CO₂.

In response to mild to moderate drought decreased stomatal conductance to save transpirational water loss is seen, while loss of ribulose biphosphate (RuBP) and thus decreased net photosynthesis dominates at severe drought (Ciais *et al.* 2005, Flexas and Medrano 2002, Penuelas *et al.* 2007). However, elevated CO₂ improves the water use efficiency via decreased stomatal conductance (Medlyn *et al.* 2001, Morison and Gifford 1984). This leads to less soil moisture depletion in elevated CO₂ (Drake *et al.* 1997, Wall *et al.* 2001) and in habitats with periodical drought enhanced CO₂ could extend the period

of active growth. Soils under grassland exposed to elevated CO₂ maintain higher moisture (Fredeen *et al.* 1997, Niklaus *et al.* 1998) and water status may strongly co-determine plant biomass responses to CO₂ enrichment (Acock and Allen 1985, Field *et al.* 1997, Gifford 1992, Koch and Mooney 1996).

Attempts to structure eco-physiological responses from plant functional types have been made (Chapin *et al.* 1996, Arft *et al.* 1999, Walker *et al.* 2006). Some studies find dwarf scrubs to be less responsive to environmental perturbations and the opportunistic grasses to be more responsive (Aerts 1995, Chapin and Shaver 1996, Graglia *et al.* 2001, Michelsen *et al.* 1999). Such differences could be due to photosynthetic capacity characteristics and carbon sink strength and also vary during growing season. However, no studies have combined a three factorial design as done here to test this hypothesis further. This study attempts to compare two functional plant types, a dwarf shrub (*Calluna vulgaris*) and a grass (*Deschampsia flexuosa*) whether responses to elevated CO₂, warming and of drought acting alone or in combination.

At the CLIMAITE field site where this study was conducted, *Calluna* and *Deschampsia* are the dominant species. *Calluna* is a slow growing evergreen scrub classed as a stress tolerant competitor (Grime *et al.* 1988) having adaptive traits for dry and nutrient low habitats f.x. xeromorphic leaves, long leaf life span, low nitrogen content, low nutrient loss and likely low photosynthetic rates (Aerts 1995). Earlier studies have shown that short term acclimation to experimental drought decreased plant water potential (Jackson *et al.* 1995), decreased light saturated net photosynthesis, transpiration and stomatal conductance, but little to no effect on WUE was seen (Gordon *et al.* 1999a, 1999b, Llorens *et al.* 2004). Grasses often have opportunistic growth patterns with rapid

leaf growth, high leaf nitrogen content (Grime *et al.* 1988) and likely high photosynthetic rates. *Deschampsia* has rhizomes (Scurfield 1954) where large nutrient pools are allocated during the wintering period ready for rapid allocation to leaf growth. Grasses often have opportunistic growth patterns with rapid leaf growth, high leaf nitrogen content (Grime *et al.* 1988) and likely high photosynthetic rates. The grass *Deschampsia* has rhizomes (Scurfield 1954) where large nutrient pools are allocated during the wintering period ready for rapid allocation to leaf growth and capacity increases. *Deschampsia* have a biphasic growth pattern (Albert *et al.* 200Xa). The first phase increases mean leaf length per shoot until maximum in June where after large proportions of leaves dies back. The second growth phase begins in August, has a maximum leaf length in September and thereafter usually cease. The maximal leaf life span is less than a year, but there are always some green leaves, giving *Deschampsia* a ‘semi-evergreen’ character (Aerts 1993). In addition *Deschampsia* has a variable number of leaf cohorts per shoot which likely allow a large degree of flexibility to initiate or extend growth in response towards environmental changes. There are a number of studies on *Deschampsia* mostly related to nutrient additions and regeneration in competition with other species (f.x. Britton *et al.* 2003, Aerts *et al.* 1990, Aerts and Chapin 2000, Alonso and Hartley 1998) and vegetative responses to PAR (Foggo and Warrington 1989, Foggo 1989).

The study is a part of the CLIMAITE experiment (Mikkelsen *et al.* 2008) in which we manipulate temperature, soil moisture and CO₂ by passive night time warming (T), periods of drought (D) and elevated CO₂ (CO₂) during the day, alone and in all combinations. We characterized both species seasonal variation in photosynthetic performance by monthly measurements of A/C_i curves and analyzed leaf nitrogen. We

reasoned that the light saturated net photosynthesis predominantly was controlled by the stomatal conductance, nitrogen content and V_{cmax} at field conditions and ambient CO_2 . However, the level of the light and CO_2 saturated photosynthesis P_{max} are not dependent of stomatal conductance and thus photosynthesis is only constrained by J_{max} . Such conditions could follow precipitation events resulting in luxurious water availability and clear sky conditions. In addition, changes in photosynthetic capacity characteristics (P_{max} and J_{max}) could be indicative of early leaf physiological adjustments which may not yet cause major impact on leaf photosynthesis level at field conditions.

We hypothesize:

- the light and CO_2 saturated net photosynthesis, P_{max} , to be controlled by the level of J_{max} and leaf nitrogen content in combination
- the light saturated net photosynthesis at ambient CO_2 to be controlled by stomatal conductance, V_{cmax} and nitrogen content in combination
- leaf nitrogen in the evergreen dwarf scrub to be stable, but variable in the grass with concurrent effects on photosynthetic performance.
- the responsiveness of photosynthetic performance to climatic changes to be less in the dwarf scrub compared to the grass
- warming (T) to advance the spring and also extend the growing season in the autumn, improving the annual photosynthetic capacity
- a summer drought (D) to decrease the photosynthetic capacity
- elevated CO_2 (CO_2) to decreases photosynthetic capacity

- responses to the climatic factors (T), (D) and (CO₂) to be additive, and hence, that there would be no interaction between climatic factors regarding their effects on photosynthetic performance.

Materials and Methods

Site and experimental setup

The experimental site is a dry heath land ecosystem in North Sealand, DK, dominated by the evergreen dwarf scrub *Calluna vulgaris* and the grass *Deschampsia flexuosa*. The experimental treatments were elevated CO₂ (CO₂), passive nighttime warming (T), summer drought (D), all combinations, (T*D), (T*CO₂), (D*CO₂), (T*D*CO₂) and un-manipulated control, replicated in six blocks in a split-plot design. Each block consisted of two octagons 8m diameter, each divided in four plots. In one octagon atmospheric CO₂ was elevated to 510 ppm with the FACE (Free Air CO₂ Enrichment, Miglietta *et al.*, 2001) technique and in the other octagon the CO₂ level was ambient. In each octagon automatized infrared reflective curtains covered one half during night, preserving a proportion of the daily incoming radiation energy, which increased the night air temperature up to 4°C. Perpendicular to the IR-curtains was a rain excluding curtain which automatically was activated by rain during the selected drought periods. In each experimental plot the temperature was measured in 2 and 10 cm depth, the soil moisture in 0-20 and 0-60 cm depth. Two climatic stations recorded precipitation, air temperature, photosynthetically active radiation and speed and wind directions. The CO₂ and warming treatments were started 3rd October 2005. The drought was started 3rd July 2006 and was continued for 5 weeks until 4th of August when soil water content reached ca. 5 % water content in the top 20 cm of the soil. For further description of site and experimental setup etc see Mikkelsen *et al.* (2008).

Leaf gas exchange

Gas exchange measurements of CO₂ and H₂O fluxes were conducted *in situ* by using two LI-6400's (LI-COR Biosciences, Lincoln, Nebraska, USA) connected to standard 2x3 cm chambers with LED light source (6400-02B) and were carried out in 2006: May 11-19th, June 12-16th, July 9-13th, August 14-21st, September 11-22nd and October 9-20th. Only healthy leaves from the top of the vegetation were selected. Pilot studies resulted in the following methodology securing highly reproducible measurements. About 10-20 *Deschampsia* leaves were positioned side by side and gently fixed by hairpins. Only healthy shoots from the top of the *Calluna* plants were selected. The *Calluna* shoots were flattened between transparent nylon strings, 0.2 mm, thick suspended in a metal frame with spacing 2.5-4 mm. The position of the shoots was upright and south facing. Leaf cuvettes were then fixed to the samples using flexible rods (Magic Arm 143, Manfrotto, Italy) fastened to the octagon scaffolds. During the following harvest care was taken to ensure that area and weight were determined on exactly the same material as inserted in the leaf cuvette.

Samples were acclimated 2-4 minutes at ambient CO₂ (380 ppm), until net photosynthesis and stomatal conductance were stabilized (± 1 CV over 30 sec). The CO₂ response curves were measured by stepping CO₂ down (from 380 ppm to 300, 200, 100, 50 ppm and then back to 380 ppm for 4 minutes re-acclimation until initial state was reached again. Then CO₂ was stepped up to saturation (from 380 ppm to 450, 510, 650, 800, 1000, 1200, 1400 ppm) at the saturating light level 1500 $\mu\text{mol photons/m}^2/\text{s}$. All using the LI-COR Auto program 'A/Ci-curve' (Settings: Min 45 and max 60 sec, reference CO₂ stable in 10 sec with CV<1%, C_i stable in 10 sec with CV<1%, IRGA matching performed at each step). Block temperature was held constant at 25°C and sample relative humidity was stabilized

at 45-55% during measurements. All measurements were area corrected. Calculation of maximum rate of Rubisco carboxylation, V_{cmax} and maximum rate of RubP regeneration, J_{max} , followed the approach of Dubois *et al.* (2007) based on the principles originating from Farquhar *et al.* (1980). The light and CO₂ saturated net photosynthesis (P_{max}) were extracted from the CO₂ response curves at reference CO₂ level, CO₂Ref = 1200 ppm CO₂.

Leaf area and nitrogen

Immediately after harvest digital pictures were taken of the leaves flattened by transparent acrylic glass beside a brick of known area. The photographs were converted to black and white and loaded into a pixel counting program (Bitmap, S. Danbæk, Department of Biology, University of Copenhagen) and the leaf areas were determined. After oven drying at 80°C the total N were determined on a Leco Truespec CN elemental determinator (Leco Corporation, Michigan, USA).

Statistics

Analyses of variance were made in SAS using the proc mixed to test for effects of the fixed factors: Month, warming (T), drought (D), elevated CO₂ (CO₂) and their interactions T*D, T*CO₂, D*CO₂, T*D*CO₂. To take into account the split-plot design, the plot was nested within octagon and octagon nested within block and these two terms set as random factors. Pre-treatment data were initially included in the full model as co-variates and handled as a fixed factor. The model were reduced by progressively dropping in-significant fixed factors, starting with the higher order interactions until only significant ($P < 0.05$) or near significant terms ($p < 0.10$) remained. Significance levels are

reported as a tendency with † when $p < 0.10$ and as significant with * when $p < 0.05$, ** when $p < 0.001$ and *** when $p < 0.0001$. All data were normal distributed and some parameters were transformed to fulfill the assumption of variance homogeneity. To interpret the direction of the significant responses we compared the least squared means. Presented parameter values are untransformed and numerical means.

Results

Climate and experimental control measurements

Snow melted in late March and from early April the mean daily temperature in 2m height increased gradually from 0°C to 25°C in late July. 2006 were the warmest year ever recorded in Denmark, 9.4°C (www.DMI.dk) and mean daily air temperatures were above 14°C in late October (Fig. 1). Warming treatment increased the temperature 1-2°C and there was 33% higher accumulated growing degree days, GDD, from 1st April to 15th May and an annual 7% increase in warmed plots compared to controls (Mikkelsen *et al.* 2008). The CO₂ concentrations in the FACE plots were elevated during daytime with monthly average daytime concentrations at 510 ppm CO₂ (Mikkelsen *et al.* 2008).

Despite some precipitation, the soil water content (SWC) decreased from 17.2 % in April to 8.8 % July in controls. This natural low SWC was extended with the experimental drought period (D) in which SWC decreased even further towards August. In this period the non-drought plots benefitted from some incidents of precipitations. Rewetting increased the SWC to 18% in August, 13.6 % in September and 16.5% in October in the controls. Soil water content in 0-20 cm depth showed decreased values in warming treatments in May, June, August and September and in response to experimental

drought in July (Fig. 2). There was a significant interaction effects D*CO₂ in July revealing increased SWC values in response to drought only when combined with elevated CO₂ (Fig. 2).

Leaf gas-exchange

The light and CO₂ saturated net photosynthesis, P_{\max} , was in May stimulated by the warming in *both species* (Fig. 3). For *Calluna* the warming increased P_{\max} and J_{\max} in June (Fig 3, Fig 5). The experimental drought and elevated CO₂ decreased the P_{\max} and J_{\max} in August. In August there was a significant interaction between experimental drought and elevated CO₂ (D*CO₂) revealing a decreased P_{\max} in the combination only (Fig. 3). In October the same (D*CO₂) interaction decreased P_{\max} and J_{\max} in the combination only (Fig. 5). Elevated CO₂ decreased the V_{\max} in June and July but increased V_{\max} in August. Experimental drought decreased V_{\max} in August and V_{\max} increased when warming and elevated CO₂ were combined, revealing the significant interaction effect (T*CO₂). In October the V_{\max} decreased when experimental drought and elevated CO₂ were combined revealing the significant interaction effect D*CO₂ (Fig. 5).

For *Deschampsia* elevated CO₂ increased the P_{\max} and J_{\max} in June and there was a tendency to increased P_{\max} and J_{\max} in response to warming, but only when combined with elevated CO₂ (T*CO₂) revealing interaction effect (Fig. 3 and Fig. 4). In July this interaction effect (T*CO₂) was reversed as P_{\max} tended to decrease and J_{\max} significantly decreased when warming and elevated CO₂ were combined. In July and August the experimental drought decreased P_{\max} and J_{\max} . In September the SWC's had equilibrated

(Fig. 2) and in the former drought plots increased P_{\max} and J_{\max} were seen. Elevated CO_2 decreased J_{\max} in August and September (Fig. 4), but in October the warming increased the P_{\max} and J_{\max} . In October there was an interaction effect $T \times \text{CO}_2$ as P_{\max} increased in response to warming only when combined with elevated CO_2 . Elevated CO_2 decreased the V_{cmax} in June, July, August, and September. In July there was an interaction effect between the former drought treatment and elevated CO_2 as V_{cmax} decreased in the combination only. Experimental drought tended to decrease V_{cmax} in August, but this effect was reversed in September where V_{cmax} significantly increased (Fig. 4).

Deschampsia leaf characteristics

The leaf characteristics of *Deschampsia* were related to the bi-phasic growth pattern, with two peaks of green biomass before and after flowering. The first phase was characterized by leaf length extension in May-June and a large dieback in July (data not shown) and the overall mean of leaf nitrogen decreased in all treatments (Fig. 6). The second phase new leaves started to sprout in August and growth continued during September and October. A burst of nitrogen was allocated to the new leaves in August and somewhat increased through September-October (Fig. 6).

The nitrogen concentration was decreased in elevated CO_2 in May, June, July and September. The experimental drought in July increased leaf nitrogen content in September and October and there was significant interaction effect between the experimental drought and elevated CO_2 in October ($D \times \text{CO}_2$) which revealed an increase in the combined plots only.

Calluna leaf characteristics

The nitrogen content in the overwintering top shoots decreased from May to July. During rewetting and in the phase of flowering in August a burst of nitrogen were built in the new formed shoots and leaf nitrogen was increasing from a new high level in August to marginally higher in September and October (Fig. 6). Leaf nitrogen was lower in response to elevated CO₂ in May, June, August, and September.

Discussion

Photosynthetic performance and elevated CO₂

The down-regulation of photosynthetic capacity via decreased maximum carboxylation rate, V_{cmax} , and the little impact on maximum velocity of RubP regeneration, J_{max} , in elevated CO₂ during most months likely is the direct impact of getting CO₂ in ‘excess’ as often reported (Ainsworth and Rogers 2007, Ainsworth and Long 2004, Drake *et al.*, 1996). In this study elevated CO₂ in general increased the light saturated net photosynthesis P_n in both species (Albert *et al.* 200Xb, 200Xc) despite the down-regulation of V_{cmax} , clearly indicating that the significant C_i increase in elevated CO₂ was most important at field conditions in line with several other studies (Ainsworth and Long 2004, Ainsworth and Rogers 2007, Drake *et al.*, 1996). In both species the response to decreasing SWC were closely followed by stomatal conductance reductions, being a major control of the P_n magnitude (Albert *et al.* 200Xa) in line with other studies on Calluna (Gordon *et al.* 1999, Llorens *et al.* 2004). In line with the above mentioned studies no general J_{max} and P_{max} decreases were seen in response to elevated CO₂. However, there were some notable responses of J_{max} and P_{max} in this study, highlighting the plasticity of the photosynthetic capacity in the grass, particularly responsive during periods with changes in SWC, and the more rigid response of the dwarf scrub as discussed below. As discussed by Ainsworth and Rogers (2007) the general pattern of down-regulations in elevated CO₂ can be explained by both reduced nitrogen supply and by reduced carbon sink strength (Drake *et al.* 1997, Rogers *et al.* 1998, Long *et al.* 2004). Both explanations are leading to appearance of a carbohydrate derived signal that can

lead to the acclimation of the photosynthetic machinery (Stitt and Krapp 1999, Long *et al.* 2004).

Photosynthetic performance affected by warming and drought

The warming treatment advanced the early growth season in both *Calluna* and *Deschampsia*. This was evident in both species, having significant higher P_{\max} and J_{\max} , and this also translated into higher light saturated photosynthesis, P_n (Albert *et al.* 200Xb, 200Xc). As the V_{cmax} was not affected by the warming treatment in early season this indicates that no carbon sink limitation of photosynthesis was apparent. This is in line with other studies demonstrating the impact of warming stimulating productivity likely via shift in phenology (Penuleas *et al.* 2007, Menzel *et al.* 2006, Jensch *et al.* 2008). This demonstrates improved photosynthetic performance in both growth forms and although rapid greening of the overwintering shoot of *Calluna* may be thought more advantageous than growth of new leaves, then these *Deschampsia* grass new leaves compensated by having all photosynthetic parameters P_{\max} , J_{\max} , V_{cmax} and P_n on a much higher level.

The plants had decreasing water availability as soil water content progressively decreased until the end of July, and the experimental drought treatment extended this period until early August. Under these conditions the stomatal conductance exerted a large degree of control of the magnitude of both species light saturated photosynthesis (Albert *et al.* 200Xa) and where *Calluna* shoots were maintained the *Deschampsia* leaves died back until rewetted in August. However, being independent of stomatal conductance the P_{\max} yield information on the capacity of the net photosynthesis and thus reveal the differences between the growth forms. Interestingly the June and not July photosynthetic

capacity via J_{\max} and P_{\max} were improved by warming for *Calluna*, but in both June and July the P_n were highest in the elevated CO_2 plots. This demonstrates the persistence of the early season stimulation of photosynthetic capacity in response to warming, but this were of little importance at ambient CO_2 conditions when increasing water shortage was occurring. This contrasts the opportunistic pattern of *Deschampsia* taking advantage of the elevated CO_2 from early on. The improved photosynthetic capacity (P_{\max} and J_{\max}) in early growth season in response to warming facilitated *Deschampsia* to synergistically increase the P_n when combined with elevated CO_2 in TCO2 treatments in May and June (Albert *et al.* 200Xb, 200Xc). However in July a large proportion of first phase *Deschampsia* leaves wilted, and warming decreased the P_{\max} and J_{\max} when combined with elevated CO_2 and also decreased the P_n and V_{cmax} . These up and down-regulations of photosynthetic performance clearly demonstrates the pronounced plasticity in this species and are likely related to water relations. Hence, the growth in the TCO2 treatments was sustained via higher stomatal conductance and water consumption in June, but ceased in July where SWC decreased to the vicinity of the wilting point. Further, negative effects of water shortage were clearly demonstrated for both species in the experimental drought treatment with the early effects in *Deschampsia*. Already in July decreased J_{\max} and P_{\max} were seen and this continued in August. The decreased P_n in July and August further demonstrated the reduced sink strength in response to experimental drought.

This highlights the opportunistic resource exploration of atmospheric CO_2 and water, which *Deschampsia* is capable of by combining pronounced photosynthetic capacity regulations with a strategy of wilting down when the water availability gets to low. In contrast, *Calluna* operates with a lower stomatal conductance and higher water

use efficiency in combination with a relatively lower photosynthetic capacity resulting in lower photosynthetic rates, but maintaining active photosynthetic metabolism throughout the drought period.

Photosynthetic performance affected by rewetting

From August and onwards frequent precipitations increased SWC and the photosynthetic performance in response to this were different for the two species. In both species the leaf nitrogen content increased significantly compared to earlier months, but where *Deschampsia* leaves appeared to continue leaf nitrogen accumulation the *Calluna* content ceased. In August both *Deschampsia* and *Calluna* were in the phase of flowering which is known to cause pronounced nitrogen allocation to the flowers (unpublished data). Likely the *Deschampsia* second phase leaves benefitted from nitrogen re-translocation from the rhizomes, but also from soil mineralization processes having its seasonal maximum at that time. In contrast *Calluna* increased its evergreen shoots length with stable nitrogen content.

For *Deschampsia* the rewetting increases in nitrogen were further stimulated in the plots previously exposed to the experimental drought, but this was not seen in *Calluna*. The increased nitrogen content is likely the reason for the higher P_{\max} , J_{\max} , P_n and V_{\max} observed. Thus the re-grown *Deschampsia* leaves in the plots previously exposed to experimental drought seems not to be carbon sink limited during this autumn. The P_{\max} was stimulated in the DCO₂ plots in October. Some early signs of senescence were seen here for *Calluna*, the J_{\max} and V_{\max} were decreased. However, this did not affect the P_{\max} and P_n significantly. This is probably demonstrating the more rigid

photosynthetic performance regulation in the evergreen *Calluna*, where the opportunistic *Deschampsia* increases photosynthetic performance immediately after rewetting. Interestingly precipitation was a major factor controlling the magnitude of *Calluna* photosynthesis across a European gradient (Llorens *et al.* 2004) and in response to warming the growth responses for *Calluna* depended on soil water availability (Penuelas *et al.* 2007). Our data supplement this and further demonstrates the influence of short term dynamics in water availability on photosynthetic performance on *Calluna* and the co-occurring *Deschampsia*.

Late season photosynthetic performance and warming

In mid-October neither leaf characteristics nor visual signs indicating senescence were observed in either species, and likely both species continued their growth further until the unusual warm autumn temperatures ceased. Thus no winter browning (Watson *et al.* 1966, Braid and Tervet 1937) were seen in *Calluna* in October, which has been suggested to be induced by occurrence of low temperature and water shortage (Jackson *et al.* 1999).

In September and October the warming treatment did not affect *Calluna* photosynthetic capacity, but warming increased *Deschampsia* J_{\max} and P_{\max} values signifying some postponement of senescence in *Deschampsia*. However, this did not cause any specific effect of warming on the P_n . Instead the overall high nitrogen content in the *Deschampsia* October leaves increased the overall level of P_{\max} and also P_n in all treatments. For the first time during season *Deschampsia* did not experience any carbon sink limitation in elevated CO_2 via decreased $V_{c\max}$ and as every other month the P_n for

Deschampsia were increased in elevated CO₂. This demonstrates the beneficial effect on photosynthetic performance caused by the extraordinary mild and warm autumn.

In contrast *Calluna* had a stable leaf nitrogen content leveling the previous months and more and more equal nitrogen content in elevated CO₂. The overall level of P_{max} and J_{max} values were increased in all treatments from September to October, demonstrating that the mild autumn affected the photosynthetic capacity of *Calluna* to a larger degree than the treatments did. Potentially the warming treatment could have increased photosynthetic capacity in October as for *Deschampsia*. And indeed, when warming was combined with elevated CO₂ the P_n were synergistically increased in *Calluna* and there were no V_{cmax} reduction indicating any carbon sink strength limitation. This demonstrates the ability for *Calluna* to benefit from the mild autumn temperatures in general, but only from the experimental warming when combined with elevated CO₂. Therefore further extension of *Calluna* productivity beyond this point is likely. Also the evergreen growth form is able to harden against frost extending the period of productivity (Caporn *et al.* 1999, Foot *et al.* 1996), and it likely preserved its investments to maintain the level of photosynthetic capacity in general, whereas the warming treatment effect on the grass likely just extended the period with high photosynthetic capacity before leaf wilting. We speculate that these effects may have shown more clearly if the warming treatment in the autumn had been more pronounced or if campaigns had been continued.

Conclusion

This study clearly demonstrates differences in the photosynthetic performance responses of the evergreen dwarf scrub *Calluna* and the opportunistic grass *Deschampsia*.

Warming during spring and early season improved the photosynthetic capacity in both species. When warming was combined with elevated CO₂ the opportunistic *Deschampsia* improved photosynthetic capacity even further concurrently with luxurious water consumption. When soil water content became unfavorable *Deschampsia* responded with accelerated leaf senescence and a major part of the leaves wilted. In contrast, the xeromorphic leaves of *Calluna* maintained metabolism with decreased photosynthetic capacity and light saturated photosynthesis in the drought period. The *Calluna* photosynthetic capacity was little responsive and only after severe drought stress the photosynthetic capacity was decreased in elevated CO₂. Rewetting caused *Deschampsia* leaves to sprout new second phase leaves with high nitrogen uptake and photosynthetic capacity, especially in the previous drought treated plots. The unusual warm autumn with frequent precipitations stimulated net photosynthesis, particular in elevated CO₂, and postponed senescence beyond October. Water relations clearly modulated the photosynthetic performance responses to elevated CO₂ and warming and have a strong impact on N-availability. The responses of the two species were much influenced by their different growth strategies.

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References

- Aerts R., Berendse F., de Caluwe H., Schmitz M. (1990)** Competition in heathland along an experimental gradient of nutrient availability. *Oikos* **57**: 310-318.
- Aerts R. (1993)** Biomass and nutrient dynamics of dominant plant species in heathlands. In: *Heathlands: Patterns and Processes in a changing environment*. Aerts R, Heil GW. (Eds.), Geobotany, 20, Kluwer Academic Publishers, pp51-84.
- Aerts R. (1995)** The advantages of being evergreen. *Tree* **10**: 402-406.
- Aerts R., Chapin III S.F. (2000)** The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research* **30**: 1-67.
- Acock B., Allen L.H. (1985)** Crop responses to elevated carbon dioxide concentrations. In: Strain BR, Cure JD (eds) *Direct effects of increasing carbon dioxide on vegetation*. Office of Energy Research, Department of Energy, Washington, pp 53-97.
- Ainsworth E.A., Long S. (2005)** What have we learned from 15 years of Free-Air CO₂ Enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New Phytologist* **165**: 351-372.
- Ainsworth E.A., Rogers A. (2007)** The response of photosynthesis and stomatal conductance to rising [CO₂]: mechanisms and environmental interactions. *Plant, Cell & Environment* **30**: 258-270.
- Ainsworth E.A., Davey P.A., Hymus G.J., Osborne C.E., Rogers A., Blum H., Nosberger J., Long S.P. (2003)** Is stimulation of leaf photosynthesis by elevated carbon dioxide concentration maintained in the long term? A test with *Lolium perenne* grown for 10 years at two nitrogen fertilization levels under Free Air CO₂ Enrichment (FACE). *Plant Cell & Environment* **26**: 705-714.
- Albert K.R., Kongstad J., Schmidt I.K., Ro-Poulsen H., Mikkelsen T.N., Michelsen A., Beier C. (200Xa)** Growth and eco-physiological characteristics of *Calluna vulgaris* and *Deschampsia flexuosa* to natural drought and warm autumn in a temperate heath. *In prepp.*
- Albert K.R., Ro-Poulsen H., Mikkelsen T.N., Michelsen A., Beier C. (200Xb)** Impact of elevated CO₂, warming and drought on photosynthesis of *Deschampsia flexuosa* in a temperate heath ecosystem. *In prepp.*
- Albert K.R., Ro-Poulsen H., Mikkelsen T.N., Michelsen A., Beier C. (200Xc)** Prolonged growing season and complex water relations in interactions of CO₂, warming and drought on *Calluna vulgaris* in a temperate heath. *In prepp.*

Alonso I., Hartley S. (1998) Effect of nutrient supply, light availability and herbivory on the growth of heather and three competing grass species. *Plant Ecology* **137**: 203–212.

Arft A.M., Walker M.D., Gurevitch J., Alatalo J. M., Bret-Harte M. S., Dale M., Diemer M., Gugerli F., Henry G.H.R., Jones M.H., Hollister R.D., Jónsdóttir I.S., Laine K., Lévesque E., Marion G.M., Molau U., Mølgaard P., Nordenhäll U., Raszhivin V., Robinson C.H., Starr G., Stenström A., Stenström M., Totland Ø., Turner P.L., Walker L.J., Webber P.J., Welker J.M., Wookey P.A. (1999) Responses of tundra plants to experimental warming: Meta-analysis of the International Tundra Experiment. *Ecological Monographs* **69**: 491-511

Atkin O.K., Tjoelker M.G. (2003) Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends in Plant Science* **8**: 343-351.

Beier C., Emmett B., Gundersen P., Tietema A., Penuelas J., Estiarte M., Gordon C., Gorissen A., Llorens L., Roda F., Williams D. (2004) Novel approaches to study climate change effects on terrestrial ecosystems in the field: Drought and passive nighttime warming. *Ecosystems* **7**: 583-597.

Bernacchi C.J., Leakey A.D.B., Heady L.E., Morgan P.B., Dohleman F.G., McGrath J.M., Gillespie K.M., Wittig V.E., Rogers A., Long S.P., Ort D.R. (2006) Hourly and seasonal variation in photosynthesis and stomatal conductance of soybean grown at future CO₂ and ozone concentrations for three years under fully open air field conditions. *Plant, Cell & Environment* **29**: 2077-2090.

Bernacchi C.J., Morgan P.B., Ort D.R., Long S.P. (2005) The growth of soybean under free air [CO₂] enrichment (FACE) stimulates photosynthesis while decreasing in vivo rubisco capacity. *Planta* **220**: 145-154.

Braid K. W., Tervet I.W. (1937) Certain botanical aspects of the dying-out of heather (*Calluna vulgaris* Hull.). *Scottish Journal of Agriculture* **20**: 1–8.

Bremer D.J., Ham J.M., Owensby C.E. (1996) Effect of elevated atmospheric carbon dioxide and open top chambers on transpiration in a tall grass prairie. *Journal of Environmental Quality* **20**: 691-701.

Britton A., Marrs R., Pakeman R., Carey P. (2003) The influence of soil-type, drought and nitrogen addition on interactions between *Calluna vulgaris* and *Deschampsia flexuosa*: implications for heathland regeneration. *Plant Ecology* **166**: 93-105.

Caporn S.J.M., Risager M., Lee J.A. (1994) Effects of atmospheric nitrogen deposition on the frost hardiness in *Calluna vulgaris*. *New Phytologist* **128**: 461-468.

Chapin III F.S., Sydonia M. B-H., Hobbie S.E., Zhong H. (1996) Plant functional types as predictors of transient responses of arctic vegetation to global change. *Journal of vegetation science* **7**: 347-358.

Chapin F.S. III, Shaver G.R. (1996) Physiological and growth responses of Arctic plants to a field experiment simulating climatic change. *Ecology* **77**: 822-840.

Classens A.T, Langeley J.A. (2005) Data-model integration is not magic: Modelling ecosystem responses to global change: Techniques and recent advances. *New Phytologist* **166**: 367-370.

Ciais P., Reichstein M., Viovy N., Granier A., Ogée J., Allard V., Aubinet M., Buchmann N., Bernhofer Chr., Carrara A., Chevallier F., De Noblet N., Friend A. D., Friedlingstein P., Grünwald T., Heinesch B., Keronen P., Knohl A., Krinner G., Loustau D., Manca G., Matteucci G., Miglietta F., Ourcival J. M., Papale D., Pilegaard K., Rambal S., Seufert G., Soussana J. F., Sanz M. J., Schulze E. D., Vesala T., Valentini R. (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature* **437**: 529-533.

Cleland E.E., Chiariello N.R., Loarie S.R., Mooney H.A., Field C.B. (2006) Diverse responses of phenology to global changes in a grassland ecosystem. *Philosophical Transactions of the National academy of Science* **103**: 13740-13744.

Curtis P.S. (1996) A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. *Plant, Cell & Environment* **19**: 127-137.

Curtis P.S. and Wang X. (1998) A meta analysis of elevated CO₂ effects on woody plant mass, form and physiology. *Oecologia* **113**: 299-313.

Christensen J.H., Christensen O.B. (2003) Climate modelling: Severe summertime flooding in Europe. *Nature* **421**: 805-806.

de Graaff M.A., van Groeningen K.J., Six J., Hungate B., van Kessel C. (2006) Interactions between plant growth and soil nutrient cycling under elevated CO₂. *Global Change Biology* **12**: 2077-2091.

Cleland, E.E., N.R. Chiariello, S.R. Loarie, H.A. Mooney & C.B. Field (2006). Diverse responses of phenology to global changes in a grassland ecosystem. *Philosophical transactions of the national academy of science* **103**: 13740-13744.

DeLucia E, Moore D and R Norby. 2005. Contrasting responses of forest ecosystems to rising atmospheric CO₂: Implications for the global C cycle. *Global Biogeochemical Cycles* **19**: GB3006.

Dermody O. (2006) Mucking through multifactor experiments; design and analysis of multifactor studies in global change research. *New Phytologist* **172**: 589-591.

Drake B., Gonzalez-Meler M., Long S. (1997) More efficient plants: a consequence of rising atmospheric CO₂? *Annual Review of Plant Physiology and Plant Molecular Biology* **48**: 609-639.

DMI.dk. Danish Meteorological Institute homepage:
http://www.dmi.dk/dmi/index/danmark/meteorologiske_ekstremer_i_danmark.htm

Dubois J.-J.B., Fiscus E.L., Booker F.L., Flowers M.D. & Reid C.D. (2007) Optimizing the statistical estimation of the parameters of the Farquhar-von Caemmerer-Berry model of photosynthesis. *New Phytologist* **176**: 402-414.

Dukes J.S., Chiariello N.R., Cleland E.E., Moore L.A., Shaw M.R., Thayer S., Tobeck T., Mooney H.A., Field C.B. (2005) Responses of grassland production to single and multiple global environmental changes. *PLoS Biology* **3**: 1829-1837.

Ellsworth D.S., Reich P.B., Naumburg E.S., Koch G.W., Kubiske M.E., Smith S.D. (2004) Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO₂ across four free-air CO₂ enrichment experiments in forest, grassland, and dessert. *Global Change Biology* **10**: 2121-2138.

Farquhar G.D., von Caemmerer S., Berry J.A. (1980) A biochemical model of photosynthetic CO₂ carbon dioxide assimilation in leaves of C₃ carbon pathway species. *Planta* **149**: 78-90.

Field C.B., Lund C.P., Chiariello N.R., Mortimer B.E. (1997) CO₂ effects on the water budget of grassland microcosm communities. *Global Change Biology* **3**: 197-206.

Finzi A.C., Allen A.S., Ellsworth D.S., DeLucia E.H., Schlesinger W.H. (2001) Litter production, chemistry and decomposition following two years of free-air CO₂ enrichment. *Ecology* **82**: 470-484.

Finzi A.C., DeLucia E.H., Hamilton J.G., Richter D.D., Schlesinger W.H. (2002) The nitrogen budget of a pine forest under free air CO₂ enrichment. *Oecologia* **132**: 567-578.

Flexas J., Medrano H. (2002) Drought-inhibition of Photosynthesis in C₃ plants: Stomatal and Non-stomatal limitations revisited. *Annals of Botany* **89**: 183-189.

Foggo M.N., Warrington I.J. (1989) Light and vegetative growth in the grasses *Deschampsia flexuosa* (L.) and *Festuca ovina* L. *Functional Ecology* **3**: 439-445.

Foggo M.N. (1989) Vegetative responses of *Deschampsia flexuosa* (L.) Trin. (Poaceae) seedlings to nitrogen supply and photosynthetically active radiation. *Functional Ecology* **3**: 337-343.

Foot J.P., Caporn S.J.M., Lee J.A., Asheden T.W. (1996) The effect of long term ozone fumigation on the growth physiology and frost hardiness on *Calluna vulgaris*. *New Phytologist* **133**: 503-511.

Fredeen A.L., Randerson J.T., Holbrook N.M., Field C.B. (1997) Elevated atmospheric CO₂ increases water availability in a water limited grassland ecosystem. *Journal of American Water Resource Association* **33**: 1033–1039.

Gifford R.M. (1992) Interaction of carbon dioxide with growth-limiting environmental factors in vegetation productivity: implications for the global carbon cycle. *Advances in Bioclimatology* **1**: 24–58.

Gordon C., Woodin S.J., Mullins C.E., Alexander I.J. (1999a) Effects of environmental change, including drought, on water use by competing *Calluna vulgaris* (heather) and *Pteridium aquilinum* (bracken). *Functional Ecology* **13**: 96-106.

Gordon C., Woodin S.J., Alexander I.J., Mullins C.E. (1999b) Effects of increased temperature, drought and nitrogen supply on two upland perennials of contrasting functional type: *Calluna vulgaris* and *Pteridium aquilinum*. *New Phytologist* **142**: 243-258.

Graglia E., Jonasson S., Michelsen A., Schmidt I.K., Havsröm M., Gustavsson L. (2001) Effects of environmental perturbations on abundance of subarctic plants after three, seven and ten years of treatments. *Ecography* **24**: 5-12.

Grime J.P., Hodgson J.G., Hunt R. (1988) Comparative plant ecology: a functional approach to common British species. London, United Kingdom, Unwin Hyman.

IPCC (2007) Climatic change 2007: The Physical Science Basis. Intergovernmental panel on Climate Change, Geneva.

Hamerlynck E.P., McAllister C.A., Knapp A.K., Ham J.M., Owensby C.E. (1997) Photosynthetic gas exchange and water relation responses of three tallgrass prairie species to elevated carbon dioxide and moderate drought. *International Journal of Plant Science* **158**: 608–616.

Harte J., Shaw R. (1995) Shifting dominance within a montane vegetation community: results of a climate warming experiment. *Science* **267**: 876-880.

Heimann M., Reichstein M. (2008) Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature* **451**: 289-292.

Hungate B.A., Holland E.A., Jackson R.B., Chapin III F.S., Mooney H.A., Field C.B. (1997) The fate of carbon in grasslands under carbon dioxide enrichment. *Nature* **388**: 576-579.

IPCC. (2001) Climatic change 2001: The Scientific Basis. Intergovernmental Panel on Climate Change, New York, Cambridge University Press.

IPCC. (2007). Climatic change 2007: The Physical Science Basis. Intergovernmental Panel on Climate Change, Geneva.

Jentsch A., Kreylling J., Boettcher-Treschow J., Beierkuhnlein C. (2008) Beyond gradual warming: extreme weather events alter flower phenology of European grassland and heath species. *Global Change Biology* **14**: 1-13.

Karnosky D.F., Mankovska B., Percy K., Dickson R.E., Podila G.K., Sôber J., Noormets A., Hendrey G., Coleman M.D., Kubiske M., Pregitzer K.S., Isebrands J.G. (1999) Effects of tropospheric O₃ on trembling aspen and interaction with CO₂: Results from an O₃-gradient and a FACE experiment. *Water, Air and Soil Pollution* **116**: 311-322.

Kelliher F.M., Leuning R., Schulze E.D. (1993) Evaporation and canopy characteristics of coniferous forests and grasslands. *Oecologia* **95**: 153–163.

King, J.S., Pregitzer, K.S., Zak, D.R., Sober, J., Isebrands, J.G., Dickson, R.E., Hendrey, G.R., Karnosky, D.F. (2001) Fine-root biomass and fluxes of soil carbon in young stands of paper birch and trembling aspen as affected by elevated atmospheric CO₂ and tropospheric O₃. *Oecologia* **128**: 237-250.

Knapp A.K., Hamerlynck E.P., Ham J.M., Owensby C.E. (1996) Responses in stomatal conductance to elevated CO₂ in 12 grassland species that differ in growth form. *Vegetatio* **125**: 31–41.

Knapp K.A., Fay P.A., Blair J.M., Collins S.L., Smidth M.D., Carlisle J.D., Harper C.W., Danner B.T., Lett M.S., McCarron J.K. (2002) Rainfall variability, carbon cycling, and Plant species diversity in a mesic grassland. *Science* **298**: 2202-2205.

Koch G.W., Mooney H.A. (1996) Response of terrestrial ecosystems to elevated CO₂: a synthesis and summary. In: Koch GW, Mooney HA (eds) Carbon dioxide and terrestrial ecosystems. Academic Press, San Diego, pp 415–429

Körner C. (2000) Biosphere responses to CO₂ enrichment. *Ecological Applications* **10**: 1590–1619.

Körner C., Asshoff R., Bignucolo O., Hättenschwiler S., Keel S.G., Peláez-Riedl S., Pepin S., Siegwolf R.T.W., Zotz G. (2005) Carbon flux and growth in mature deciduous forest trees exposed to elevated CO₂. *Science* **309**: 1360 – 1362.

Körner C. (2006) Plant CO₂ responses: An issue of definition, time and resource supply. *New Phytologist* **172**: 393-411.

Lauber I.R., Körner C. (1997) *In situ* stomatal response to long term CO₂ enrichment in calcareous grassland plants. *Acta Oecologia* **18**: 221-229.

Leuzinger S., Körner C. (2007) Water savings in mature deciduous forest trees under elevated CO₂. *Global Change Biology* **13**: 2498–2508.

Liberloo M., Tulva I., Raïm O., Kull O., Ceulemans (2007) Photosynthetic stimulation under long-term CO₂ enrichment and fertilization is sustained across a closed *Populus* canopy profile (EUROFACE). *New Phytologist* **173**: 537-549.

Llorens L., Penuelas J., Beier C., Emmett B.A., Estiarte M., Tietema A. (2004) Effects of an experimental increase of temperature and drought in the photosynthetic performance of two ericaceous shrub species along a north–south European gradient. *Ecosystems* **7**: 613–624.

Long S.P., Drake B.G. (1992) Photosynthetic CO₂ assimilation and rising CO₂ concentrations. In Crop photosynthesis spatial and temporal determinants (eds. Baker N.R. and Thomas H.), pp 69-103, Elsevier, Amsterdam, The Netherlands.

Long S.P., Ainsworth E.A., Bernacchi E.A., Davey P.A., Hymus G.J., Leakey A.D.B., Morgan P.B., Osborne C.P. (2006a) Long term responses of photosynthesis and stomata to elevated CO₂ in managed systems. In Managed Ecosystems and CO₂. Case studies, Processes and Perspectives (eds Nösberger J., Long S.P., Norby R.J., Stitt M., Hendry G.R., Blum H), pp 253-270, Springer-Verlag, Heidelberg, Germany.

Long S.P., Ainsworth E.A., Leakey A.B.D., Nosberger J. Ort D.R. (2006b) Food for thought: lower than expected crop yield stimulation with rising CO₂ concentrations. *Science* **312**: 1918-1921.

Long S.P., Ainsworth E.A., Rogers A., Ort D.R. (2004) Rising atmospheric carbon dioxide: plants FACE the future. *Annual review of plant biology* **55**: 591-628.

Lou Y., Wan S., Hui D., Wallace L.L. (2001) Acclimatization of soil respiration to warming in a tall grass prairie. *Nature* **413**: 622-625.

Lou Y. (2007) Terrestrial carbon cycle feedback to climate warming. *Annual Review of Ecology, Evolution and Systematics* **38**: 683-712.

Lou Y., Su B., Currie W.S., Dukes J.S., Finzi A., Hartwig U., Hungate B., McMurtrie R.E., Oreon R., Parton W.J., Pataki D.E, Shaw M.R., Zak D.R, Field C.B. (2004) Progressive Nitrogen Limitation of Ecosystem Responses to Rising Atmospheric Carbon Dioxide. *Bioscience* **54**: 731-739.

Medlyn B.E., Barton C.V.M., Broadmeadow M.S.J., Ceuleman R., De Angelis P., Forstreuter M., Freeman M., Jackson S.B., Kellomaki S., Laitat E., Rey A., Roberntz P., Sigurdsson B.D., Strassmeyer J., Wang K., Curtis P.S., Jarvis (2001)

Stomatal conductance of forest species after long-term exposure to elevated CO₂ concentration: a synthesis. *New Phytologist* **149**: 247–264.

Menzel A., Fabian P. (1999) Growing season extended in Europe. *Nature* **397**: 659.

Menzel A., Sparks T.H., Estrella N., Koch E., Aasa A., Ahas R., Alm-Kûbler K., Bissolli P., Braslavska O., Briede A., Chielewski F.M., Crepensek Z., Curnel Y., Dahl Å., Defila C., Donnelly A., Filella Y., Jatzczak K., Måge F., Mestre A., Nordli Ø., Penuelas J., Pirinen P., Remosova V., Scheifinger H., Striz M., Susnik A., van Vliet A.J.H., Wielgolaski F.-E., Zach S., Züst A. (2006) European phenological response to climate change matches the warming pattern. *Global Change Biology* **12**: 1969–1976.

Michelsen A., Graglia E., Schmidt I.K., Jonasson S., Sleep D., Quarmby C. (1999) Differential grass and dwarf shrub responses to long term changes in soil microbial biomass C, N, and P by factorial NPK fertilizer fungicide and labile carbon addition to a heath. *New Phytologist* **143**: 523–538.

Miglietta F., Peressotti A., Vaccari F.P., Zaldei A., deAngelis P., Scarascia-Mugnozza, G. (2001) Free-air CO₂ enrichment (FACE) of a poplar plantation: the POPFACE fumigation system. *New Phytologist* **150**: 465–476.

Moore B.D., Cheng S-H., Sims D., Seemann J.R. (1999) The biochemical and molecular basis for photosynthetic acclimation to elevated atmospheric CO₂. *Plant Cell & Environment* **22**: 567–582.

Morison J.I.L., Gifford R.M. (1984) Plant growth and water use with limited water supply in high CO₂ concentrations. II. Plant dry weight, partitioning and water use efficiency. *Australian Journal of Plant Physiology* **11**: 375–384.

Morgan J.A., Pataki D.E., Körner C., Clark H., Del Grosso S.J., Grünzweig J.M., Knapp A.K., Mosier A.R., Newton P.C.D., Niklaus P.A., Nippert J.B., Nowak R.S., Parton W.J., Polley H.W., Shaw M.R. (2004) Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO₂. *Oecologia* **140**: 11–25.

Niklaus P.A., Spinnler D., Körner C. (1998) Soil moisture dynamics of calcareous grassland under elevated CO₂. *Oecologia* **117**: 201–208.

Norby R.J., Lou Y. (2004) Evaluating ecosystem responses to rising atmospheric CO₂ and global warming in a multi-factor world. *New Phytologist* **162**: 281–293.

Nowak R.S., Ellsworth D.S., Smith S.D. (2004) Functional responses of plants to elevated atmospheric CO₂ – do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytologist* **162**: 253–280.

Oren R., Ellsworth D.S., Johnsen K.H., Phillips N., Ewers B.E., Maier C., Schäfer K.V.R., McCarthy H., Hendry G., McNulty S.G., Katul G.C. (2001) Soil fertility limits carbon sequestration by forest ecosystems in a CO₂ enriched atmosphere. *Nature* **411**: 469-472.

Parry M.A.J., Andralojc P.J., Mitchell R.A.C., Madgwick P.J., Keys A.J. (2003) Manipulation of Rubisco: the amount, activity, function and regulation. *Journal of Experimental Botany* **54**: 1321-1333.

Poorter H., Vandeijver CADM, Boot RGA, Lambers H. 1995. Growth and carbon economy of a fast-growing and a slow-growing grass species as dependent on nitrate supply. *Plant and Soil* **171**: 217-227.

Penuelas J. and Filella I. (2001) Responses to a warming world. *Science* **294**: 793-795.

Peñuelas J., Prieto P., Beier C., Cesaraccio C., de Angelis P., de Dato G., Emmett B.A., Estiarte M., Garadnai J., Gorissen A., Lang E.K., Kröel-Dulay G., Llorens L., Pellizzaro G., Riis-Nielsen T., Schmidt I.K., Sirca C., Sowerby A., Spano D., Tietma A. (2007) Response of plant species richness and primary productivity in shrublands along a north-south gradient in Europe to seven years of experimental warming and drought: reductions in primary productivity in the heat and drought year of 2003. *Global Change Biology* **13**: 2563-2581.

Reich P.B., Hobbie S.E., Lee T., Ellsworth D.S., West J.B., Tilman D., Knops J.M.H., Naeem S., Trost J. (2006) Nitrogen limitation constrains sustainability of ecosystem response to CO₂. *Nature* **440**: 922-925.

Robredo A., Pérez-López U., Sainz de la Maza H., González-Moro B., Lacuesta M., Mena-Petite A., Munoz-Rueda A. (2007) Elevated CO₂ alleviates the impact of drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis. *Environmental and Experimental Botany* **59**: 252-263.

Rogers A., Ainsworth E.A. (2006) The response of foliar carbohydrates to elevated carbon dioxide concentration. In *Managed Ecosystems and CO₂. Case Studies, Processes and Perspectives* (eds. Nösberger J., Long S.P., Norby R.J., Stitt M., Hendrey G.R., Blum H.) pp 293-308. Springer Verlag, Heidelberg, Germany.

Rogers A., Fischer B.U., Bryant J., Frehner M., Blum H., Raines C.A., Long S.P. (1998) Acclimation of photosynthesis to elevated CO₂ under low-nitrogen nutrition is affected by the capacity for assimilate utilization. Perennial ryegrass under free air CO₂ enrichment. *Plant Physiology* **118**: 683-689.

Rogers A., Allen D.J., Davey P.A., Morgan P.B., Ainsworth E.A., Bernacchi C.J., Cornic G., Dermody O., Dohleman F.G., Heaton E.A., Mahoney J., Zhu X.G., Delucia E.H., Ort D.R., Long S.P. (2004) Leaf photosynthesis and carbohydrate

dynamics of soybeans grown throughout their life-cycle under Free-Air Carbon dioxide enrichment. *Plant Cell & Environment* **27**: 449-458.

Rogers A., Ainsworth E.A., Kamman C. (2006) Perspectives of the future of free air CO₂ enrichment studies. In *Managed Ecosystems and CO₂. Case studies, Processes and Perspectives* (eds Nösberger J., Long S.P., Norby R.J., Stitte M., Hendrey G.H., Blum H.), pp 431-450, Springer-Verlag, Heidelberg, Germany.

Rustad L., Campbell J., Marion G., Norby R., Mitchell M., Hartley A., Cornelissen J., Gurevitch J., GCTE-NEWS. (2001) A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* **126**: 543–562.

Rustad L.E. (2006) From transient to steady state response of ecosystems to atmospheric CO₂-enrichment and global climate change: Conceptual challenges and need for an integrated research. *Plant Ecology* **182**: 43-62.

Rustad L.E. (2008) The response of terrestrial ecosystems to global climate change: Towards an integrated approach. *Science of the total environment* **404**: 222-235.

Scurfield G. (1954) Biological Flora of the British Isles, *Deschampsia flexuosa* (L.) Trin. *Journal of Ecology* **42**: 225–233.

Shaver G.R., Canadell J., Chapin FS III, Gurevitch J., Harte J., Henry G., Ineson P., Jonasson S., Melillo J, Pitelka L., Rustad L. (2000) Global warming and terrestrial ecosystems: a conceptual framework for analysis. *Bioscience* **50**: 871-882.

Shaw M.R., Zavaleta E.S., Chiariello N.R., Cleland E.E., Mooney H.A., Field C.B. (2002) Grassland responses to global environmental changes suppressed by elevated CO₂. *Science* **298**: 1987-1990.

Springer J.C., Ward J.K. (2007) Flowering time and CO₂. *New Phytologist* **176**: 243-255.

Teng N., Wang J., Chen T., Wu X., Wang Y., Lin J (2006) Elevated CO₂ induces physiological, biochemical and structural changes in leaves of *Arabidopsis thaliana*. *New Phytologist* **172**: 378-378.

Turnbull M.H., Tissue D.T., Murthy R., Wang X., Sparrow A.D., Griffin K.L (2004) Nocturnal warming increases photosynthesis at elevated CO₂ partial pressure in *Populus deltoides*. *New Phytologist* **161**: 819-826.

Turnbull M.H., Murthy R., Griffin K.L. (2002) The relative impacts of daytime and night time warming on photosynthetic capacity in *Populus deltoides*. *Plant Cell & Environment* **25**: 1729-1737.

Volk M., Niklaus P.A, Körner C. (2000) Soil moisture effects determine CO₂ responses of grassland species *Oecologia* **125**: 380–388.

Wall G.W., Brooks T.J., Adam N.R., Cousins A., Triggs J., Kimball B.A., Pinter P.J. Jr., LaMorte R.L., Ottman M.J., Leavitt S.W., Matthias A.D., Williams D.G., Webber A.N. (2001) Leaf photosynthesis and water relations of grain sorghum grown in free-air CO₂ enrichment (FACE) and water stress. *New Phytologist* **152**: 231-248

Walther G-R., Post E., Convey P., Menzel A., Parmesan C., Beebee T. J. C., Fromentin J-M., Hoegh-Guldberg O., Bairlein F. (2002) Ecological responses to recent climate change. *Nature* **416**: 389-395.

Wan S., Hui D., Wallace L., Lou Y. (2005) Direct and indirect effects of experimental warming on ecosystem carbon processes in a tall grass prairie. *Global Biogeochemical Cycles* **19**: GB2014. Doi:10.1029/2004GB002315.

Figure 1: Seasonal variation in climate parameters. (Top) Shown are the daily averages of soil water content in control plots in 0-20 cm and 0-60 cm depth and daily accumulated precipitation, (Middle) daily averages of air temperature in 2 m height and (Bottom) photosynthetically active radiation (PAR).

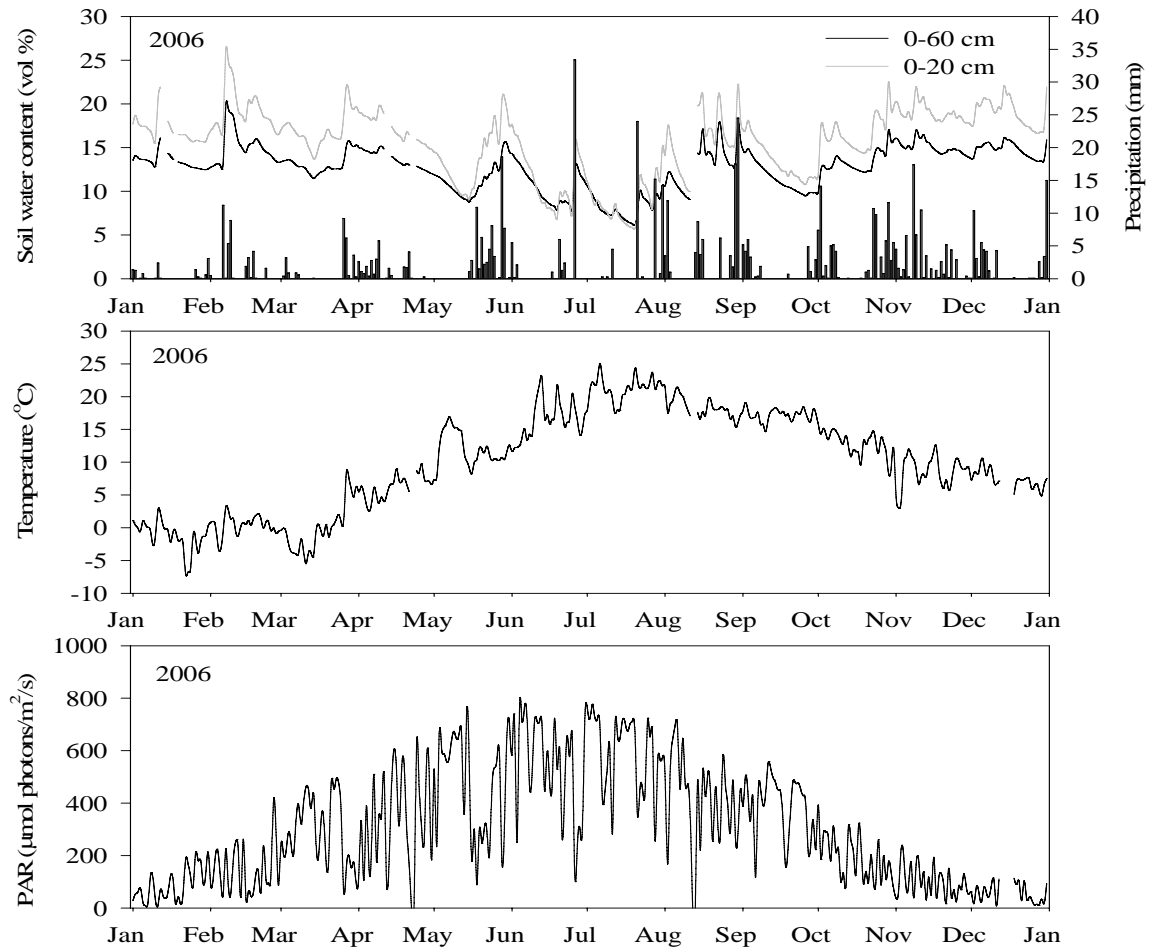


Figure 2: Soil water content in 0-20 cm depth (SWC) during each gas-exchange campaign. Shown are the mean \pm standard errors. Treatments are (A) un-manipulated control, (T) passive nighttime warming, (D) experimental drought, active in July- early August, (TD) warming and drought combined, (CO₂) elevated CO₂, (TCO₂) warming and elevated CO₂ combined, (DCO₂) drought and elevated CO₂ combined, (TDCO₂) warming, drought and elevated CO₂ combined. Statistically significant factors and interactions are shown with treatment letters and levels of significance with *** if $p < 0.0001$, ** if $p < 0.001$, * if $p < 0.05$ and † if $p < 0.10$.

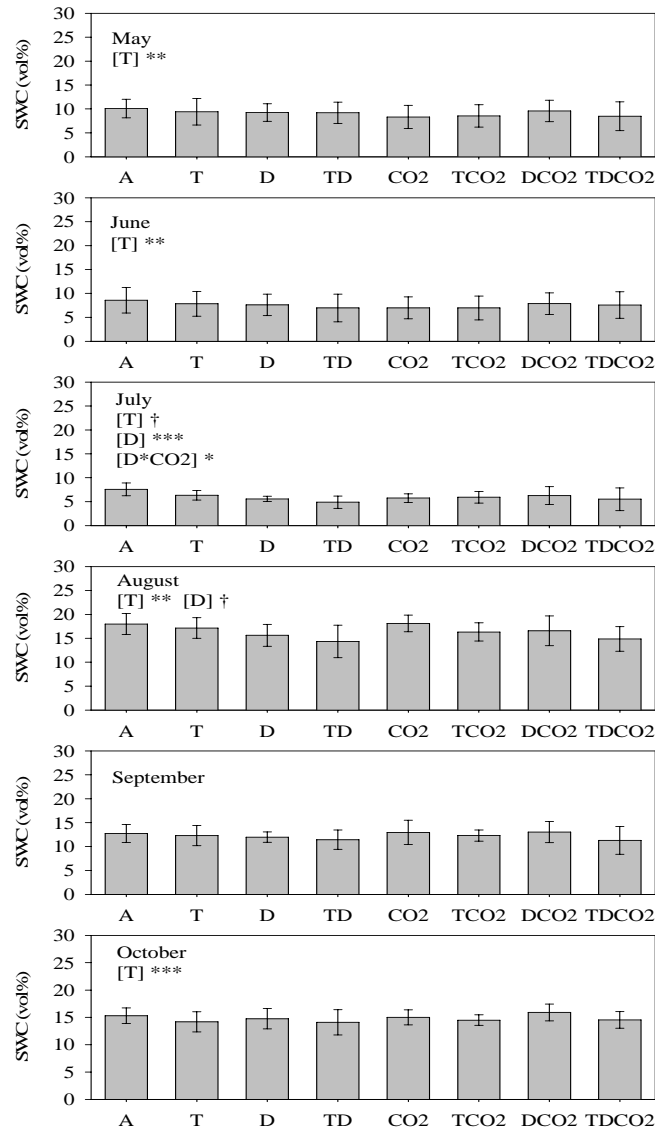


Figure 3: Light and CO₂ saturated net photosynthesis, P_{\max} . Left panels are *Calluna* and right panes are *Deschampsia*. Shown are the mean \pm standard errors. Treatments abbreviations and statistics as in figure 2.

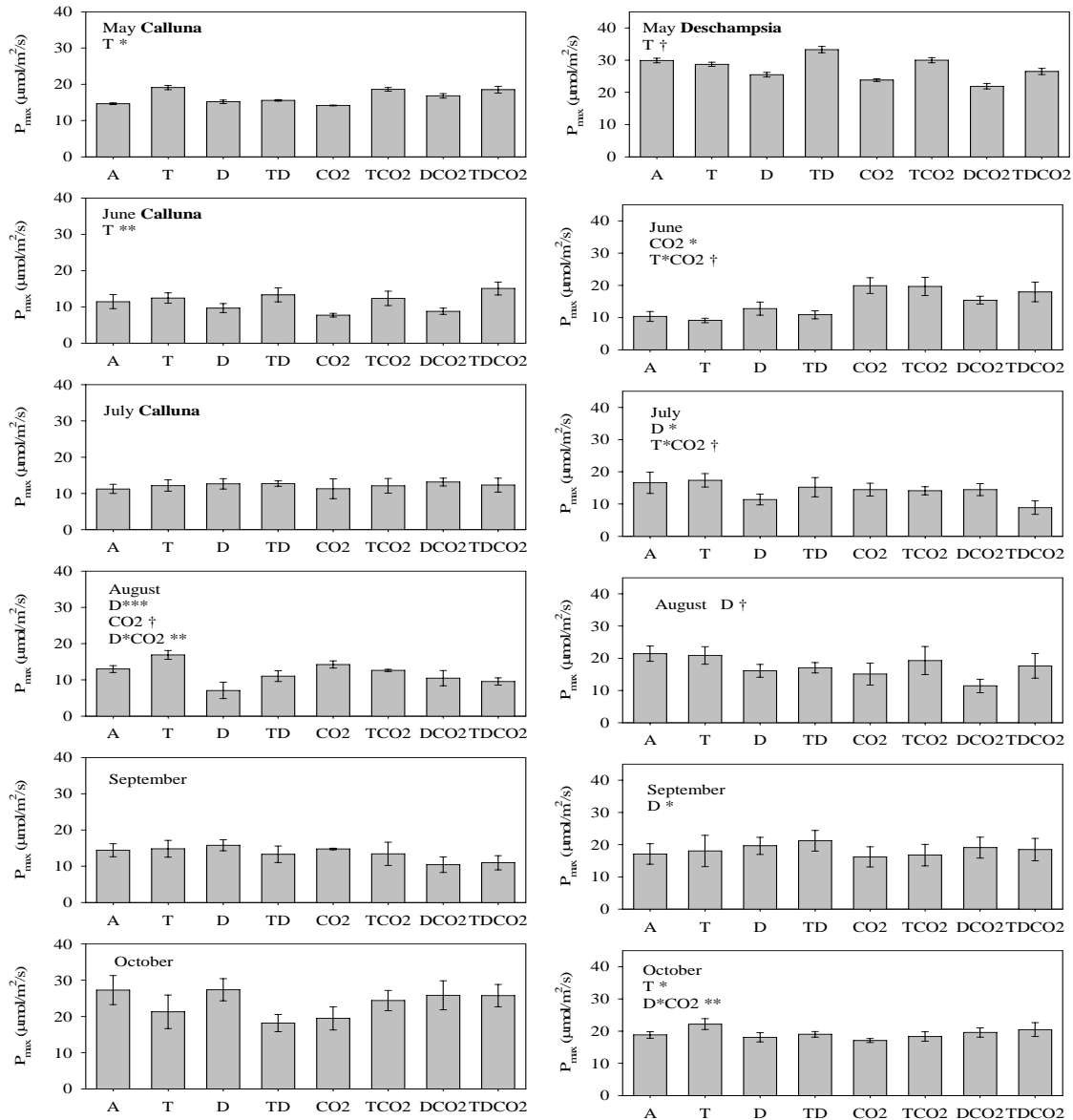


Figure 4: *Deschampsia* J_{\max} and V_{\max} . Left panels are Maximal velocity of Rubisco regeneration, J_{\max} . Right panels are Maximal velocity of Rubisco carboxylation, V_{\max} . Shown are the mean \pm standard errors. Treatments abbreviations and statistics as in figure 2.

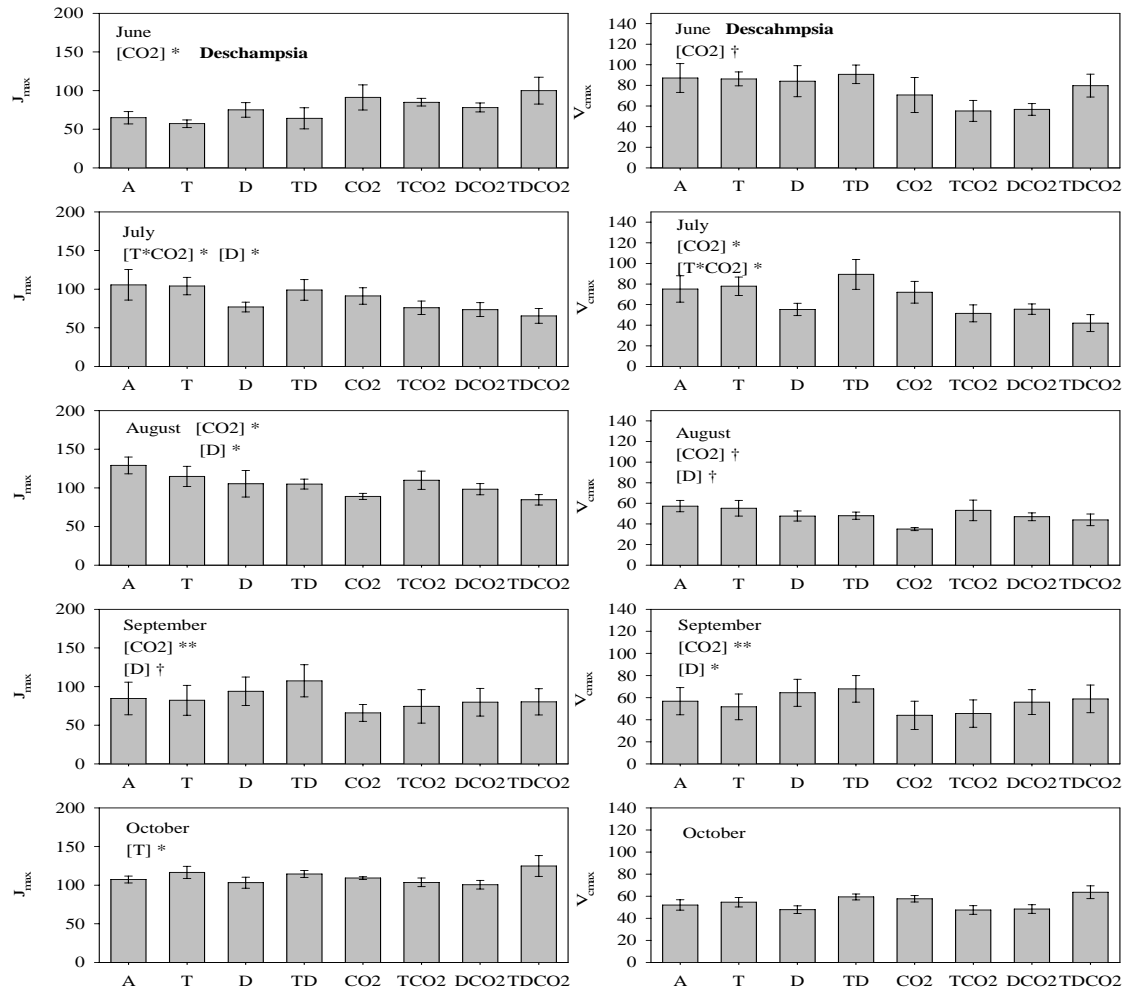


Figure 5: *Calluna* J_{\max} and V_{\max} . Left panels are Maximal velocity of Rubisco regeneration, J_{\max} . Right panels are Maximal velocity of Rubisco carboxylation, V_{\max} . Shown are the mean \pm standard errors. Treatments abbreviations and statistics as in figure 2.

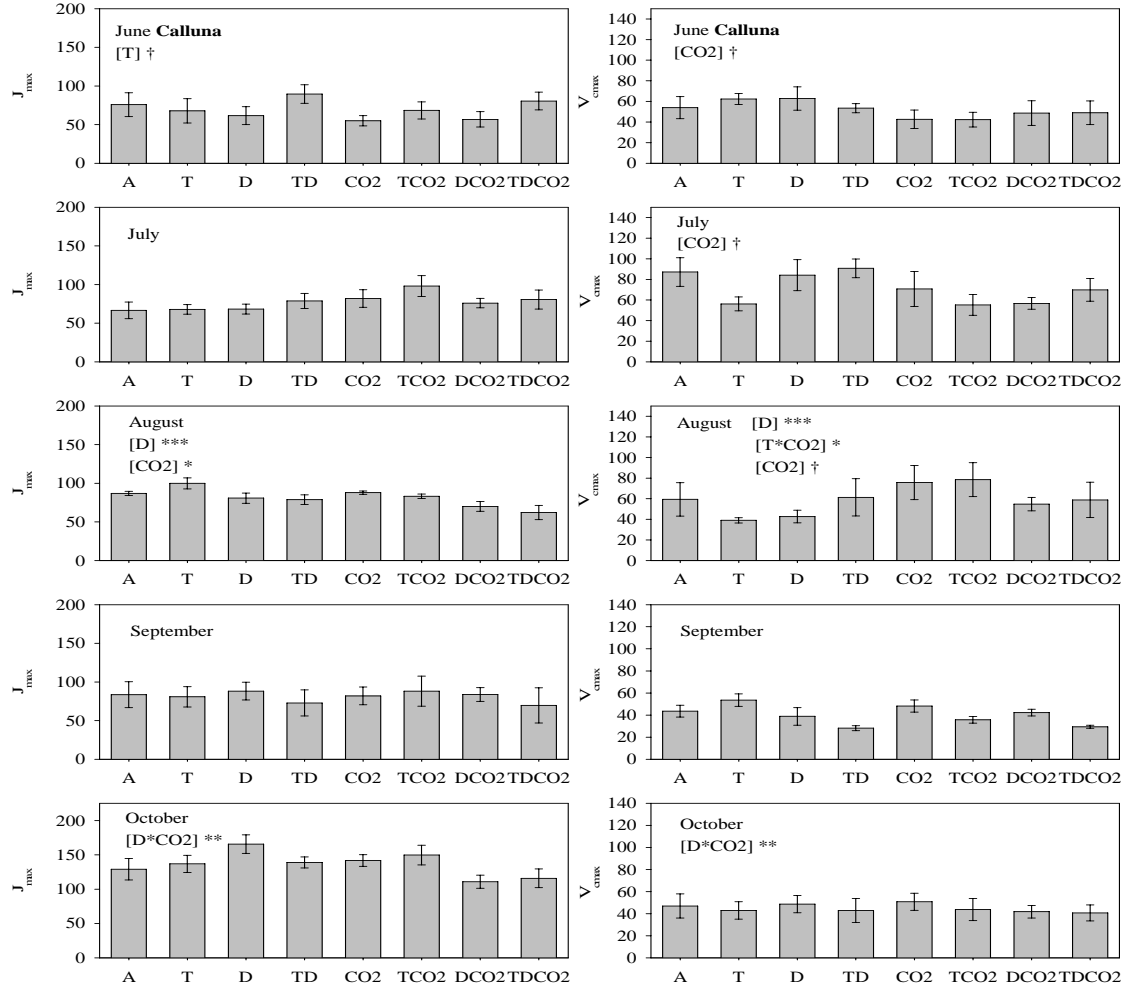
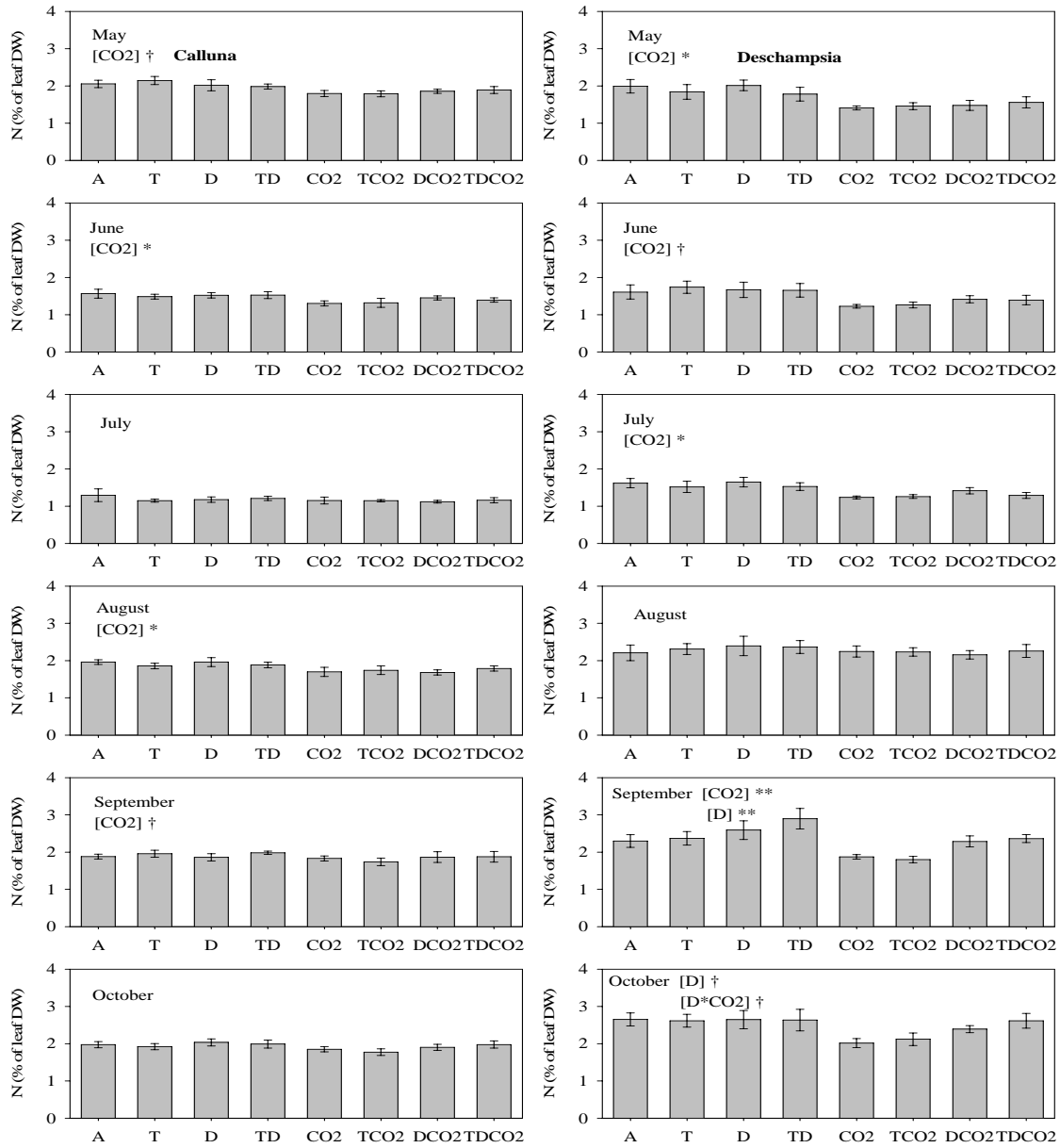


Figure 6: Leaf Nitrogen content. Left panels are *Calluna*. Right panels *Deschampsia*. Shown are the mean \pm standard errors. Treatments abbreviations and statistics as in figure 2.



Paper V

Ambient UV-B radiation decreases photosynthesis in high arctic *Vaccinium uliginosum*.

Kristian Rost Albert, Helge Ro-Poulsen, Teis Nørgaard Mikkelsen (2008)

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Ambient UV-B radiation decreases photosynthesis in high arctic *Vaccinium uliginosum*

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An UV-B-exclusion experiment was established in high arctic Zackenberg, Northeast Greenland, to investigate the possible effects of ambient UV-B on plant performance. During almost a whole growing season, canopy gas exchange and Chl fluorescence were measured on *Vaccinium uliginosum* (bog blueberry). Leaf area, biomass, carbon, nitrogen and UV-B-absorbing compounds were determined from a late season harvest. Compared with the reduced UV-B treatment, the plants in ambient UV-B were found to have a higher content of UV-B-absorbing compounds, and canopy net photosynthesis was as an average 23% lower during the season. By means of the JIP-test, it was found that the potential of processing light energy through the photosynthetic machinery was slightly reduced in ambient UV-B. This indicates that not only the UV-B effects on PSII may be responsible for some of the observed reduction of photosynthesis but also the effects on other parts of the photosynthetic machinery, e.g. the Calvin cycle, might be important. The 60% reduction of the UV-B irradiance used in this study implies a higher relative change in the UV-B load than many of the supplemental experiments do, but the substantial effect on photosynthesis clearly indicates that *V. uliginosum* is negatively affected by the current level of UV-B.

Introduction

Stratospheric ozone depletion increases UV-B radiation (280–315 nm) in the biosphere (Madronich et al. 1998), and although this may be mitigated by increased cloudiness, it has raised concerns about UV-B impacts on plants (Caldwell et al. 1998, Rozema et al. 1997; for a critical view, see Allen et al. 1998). The UV-B exposure in the Arctic region is currently considered to be near the maximum, and the ozone column is predicted to recover towards the middle of the century (WMO 2003). Furthermore, not only the relative increase in UV-B irradiance has been occurring most rapidly at high latitudes but also the absolute net depletion of ozone has been highest, resulting in the potentially highest impact on the

vegetation there (Björn et al. 1999, Paul 2001). Moreover, arctic plants are exposed to extreme living conditions with respect to climate and the limit of their distribution. Also, the longevity of many arctic plants makes them adapt only slowly to environmental changes (Callaghan and Jonasson 1995). Thus, additional stress factors of importance may affect arctic plants negatively.

Arctic plants have in general been shown to be susceptible to increases in incident UV-B (Johanson et al. 1995, Gehrke et al. 1996), but few reports from exclusion studies addressing the impact on vegetation are available from the Arctic (Albert et al. 2005, Bredahl et al. 2004, Phoenix et al. 2002, Rinnan et al. 2005). Some

Abbreviations – CS, cross-section; RC, reaction centre.

longer term UV-exclusion studies have been conducted in South America (Robson et al. 2003, Rousseaux et al. 1999), Antarctic (Day et al. 2001, Xiong and Day 2001) and elsewhere. In the Arctic, UV reduction resulted in increased flowering and berry production and a decrease in UV-B-absorbing compounds in sub-Arctic dwarf scrubs (Phoenix et al. 2002). UV reduction in high Arctic studies, although of short duration, resulted in increased F_v/F_m in *Salix arctica* and *Vaccinium uliginosum* and reduced the stomatal conductance and internal CO_2 concentration in *Salix* (Bredal et al. 2004). Moreover, by optimizing the natural irradiance by leaf angle control, UV reduction was observed to have positive impacts on almost all measured and derived fluorescence parameters on *S. arctica*, i.e. F_v/F_m and PI indexes (Albert et al. 2005).

Several studies have shown that PSII is sensitive to UV-B (Melis et al. 1992, Strid et al. 1990). However, other studies (Allen et al. 1997, Nogués and Baker 1995) have questioned whether PSII damage is the primary cause of reduction in photosynthesis. It seems that UV-B-induced reduction in photosynthesis can occur prior to, or in absence of, depressions in PSII function and probably involves impairments in the Calvin cycle (Allen et al. 1999, Nogués and Baker 1995). Because UV-B screening pigments such as flavonoids (Caldwell and Flint 1994, van de Staaij et al. 1995) and hydroxycinnamates (Burchard et al. 2000) to some degree do protect against deleterious effects of UV-B (Alenius et al. 1995), they probably influence photosynthetic processes in underlying tissues.

This experiment aimed at investigating whether the photosynthetic performance of high arctic vegetation would improve if UV-B irradiance were reduced. The manipulative approach chosen was to reduce the UV-B radiation load on the vegetation by means of filters to assess effects of present-day UV-B radiation. This approach excludes the spectral matching problems of natural irradiation experienced in many earlier UV-B supplementation experiments (Flint et al. 2003). A robust measure of photosynthesis was obtained by canopy-level CO_2 gas exchange measurements. In addition, the processing of light energy through PSII was measured by recording transients of Chl *a* fluorescence using the JIP-test and associated parameters (Strasser et al. 2004), a methodology discussed by Lazár (2006), Strasser et al. (2000) and Strasser et al. (2004).

It is hypothesized that (1) more UV-B-absorbing compounds are found at ambient compared with the reduced UV-B level and (2) PSII performance (i.e. estimated electron transfer and performance indexes) is higher at reduced UV-B with (3) possible improvements of the photosynthetic performance.

Materials and methods

Experimental site

The fieldwork was carried out in a high arctic heathland at Zackenberg Research Station, Northeast Greenland (74°30' N, 21°E), during July and August 2002. The plant species investigated was the long-lived deciduous dwarf shrub *V. uliginosum* L., ssp. *microphyllum* Lge., dominating the vegetation cover in the experimental area. High temperatures induced a fast snowmelt beginning in early June, resulting in snowfree vegetation from mid-June. Leaves were fully developed and expanded when the experiments were initiated. Senescence period began early August after a period of cool weather from late July, which continued throughout August (Rasch and Canning 2003).

Experimental set-up and treatments

The aim was to establish plots where parts of the UV spectrum in natural daylight were reduced by filtering the solar radiation through a Mylar® film (type D, DuPont Teijin Films, Wilmington, DE). In general, the Mylar filter transmits $\lambda > 320$ nm (Cybulski and Peterjohn 1999). A Teflon® filter (Fluoretek AB, Knivsta, Sweden), which is transparent to UV, was used as control. In general, it transmits $\lambda > 280$ nm (Cybulski and Peterjohn 1999). Measurements in the experimental area with a broadband cosine corrected UV-B sensor (UV-S-310-T; Scintec, Atmosphärenmesstechnik GmbH, Tübingen, Germany – now manufactured as UV-S-B-T by Kipp and Zonen B.V., Delft, The Netherlands) showed that the plant canopy under the Teflon filter was exposed to about 91% of incoming UV-B but only to 39% under the Mylar filter, with some variation depending on the exposure angle to the sun. PAR was reduced to 97% under the Teflon filter and 89% under the Mylar filters compared with open plots (Bredal et al. 2004). No deterioration of the filters was detected after exposure to field conditions (Bredal et al. 2004). In the following, Teflon is referred to as 'ambient UV-B' and Mylar as 'reduced UV-B'.

Individual *Vaccinium* plants were selected, and around each plant, circular 13.5-cm-diameter metal chamber bases were inserted approximately 5 cm into the soil to enable CO_2 flux measurements. Above the bases, the filters were placed parallel to the soil surface approximately 10 cm above the canopy supported by 30 × 30 cm aluminium frames. The plants were watered with 0.25 l per chamber base three times within the first week to ensure fine root reestablishment. This did not change soil moisture conditions between treatments. The species composition within the plots was totally

dominated by *Vaccinium* plants (>95%) and mosses, with some graminoids occurring in a few plots.

The experiment was a randomized design with 10 experimental plots beneath Mylar filter and 10 plots below Teflon filter. Measurements of Chl *a* fluorescence, gas exchange and microclimate were performed approximately every fourth day during approximately 4 h between 14:30 and 19:30 h.

Chl *a* fluorescence and JIP-test

Leaves were dark adapted for minimum 25 min before transients of Chl *a* fluorescence were measured with a Handy PEA (Hansatech Instruments Ltd., King's Lynn, Norfolk, UK) at 650 nm light with an intensity of $2500 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Tsimilli-Michael and Strasser 2001). Measurements were performed on detached leaves in situ using a pincer to place excised leaves in dark adaptation clips. To avoid stripping of the canopy, five healthy top leaves of random leaf angle were sampled just outside the chamber base but still well within the filter covered area. For possible comparison of fluorescence and gas exchange parameters, the fluorescence recordings were initiated approximately at the same time as gas exchange measurements were taken.

From the fluorescence transient, the measured parameters ($F_o = F_{50\mu\text{s}}$, $F_K = F_{300\mu\text{s}}$, $F_J = F_{2\text{ms}}$, $F_I = F_{30\text{ms}}$, $F_M = F_P \cdot tF_M$ and Area) lead to calculation and derivation of a range of new parameters according to Strasser et al. (2004). See also Albert et al. (2005) for a summary of all parameters and formulae. Briefly, the careful translation of the measured parameters into JIP-test parameters provides information on the stepwise flow of energy through PSII at different levels: (1) specific fluxes on the level per reaction centre (RC) and these are for absorption (ABS/RC), trapping (TR_o/RC), dissipation (DI_o/RC) and electron transport (ET_o/RC) and (2) phenomenological fluxes on the level of the excited leaf cross-section (CS), and these are for absorption (ABS/CS), trapping (TR_o/CS), dissipation (DI_o/CS) and electron transport (ET_o/CS). These fluxes are interrelated and outlined in Fig. 1 as pipeline models with specific fluxes in the membrane model and the phenomenological fluxes in the leaf model. The JIP-test proposes equations to convert experimental fluorescence signals into biophysical or bioenergetic meaning (similar to Beer–Lambert's law, which transforms the fraction of transmitted light *T* into chemical concentration). Ideally, this is performed by measures of reflection or absorption or alternatively it is approximated by, i.e., fluorescence data. This approximation is performed by assuming that either F_o or F_M are reasonable measures of the absorption energy flux per excited CS of leaf sample (ABS/CS) in arbitrary units of

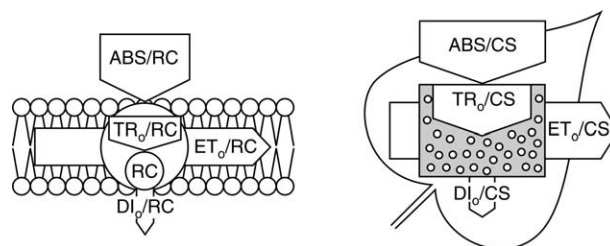


Fig. 1. Pipeline models. Left: The membrane model displaying specific fluxes per PSII RC. Right: The leaf model with phenomenological fluxes per leaf CS. See Materials and methods for JIP-test parameter presentation. Figures are modified with permission from Strasser et al. (2000).

a particular leaf sample in the dark-adapted state and then the phenomenological fluxes can be estimated. The specific and phenomenological fluxes are interrelated by the quantum efficiencies, which are (1) the maximum quantum yield (with all PSII RCs open) of primary photochemistry (F_v/F_M), which in this terminology is equal to the efficiency by which an absorbed photon will be trapped by the PSII RC with the resultant reduction of Q_A to Q_A^- (TR_o/ABS), and (2) the efficiency by which a trapped exciton, having triggered the reduction of Q_A to Q_A^- can move an electron further than Q_A^- into the intersystem electron transport chain (ET_o/TR_o). Integrative parameters, so-called performance indexes, reflecting performance of the overall energy flow processing are (1) based on quantum efficiencies and hereby related to the situation of assuming equal absorption (PI_{ABS}) and (2) based on the phenomenological fluxes related to the per leaf CS level (PI_{CS_o} and PI_{CS_m}). Estimation of the density of active PSII reaction centres per leaf CS (RC/CS_M) is also possible. Brief parameter descriptions are given in the Results section the first time each parameter occurs.

Gas exchange

Gas exchange was measured (CIRAS-1; PP systems, Hitchin, UK) and connected to an open system ventilated canopy chamber with an internal volume of 3 l (CPY-3 TPX and Stainless Steel ring; PP Systems, Hertfordshire, UK). The in-flow rate was 11 l min^{-1} as compared with an analysis sample rate at 300 ml min^{-1} . The transparent chamber was in place for 2–5 min before five successive readings were taken. The chamber was then darkened with a light-excluding wooden box and five more successive readings were then taken after a 5- to 8-min period.

At each measuring day during the season, a total of 50 leaves were collected from each treatment. Leaf area and dry weight were determined, and then correlations were calculated for each treatment. At the end of the season, the plots were harvested and the dry weight of leaves and aboveground stems in each plot was determined. Leaf

area in each plot was then estimated from the correlation between leaf area and leaf weight for each treatment. During whole season, no significant differences in area and dry weight of the leaves were observed.

The gas exchange was calculated as CO_2 flux ($\mu\text{mol m}^{-2} \text{s}^{-1}$) = $\Delta\text{CO}_2 (V \times 1000)/(A \times 22.414 \times 1000 \times 60)$, where ΔCO_2 is the difference of CO_2 concentration between the air supply and the chamber air in parts per million, V is the flow measured in ml min^{-1} at STP through the chamber, A is the canopy leaf area (cm^2) estimated from the final harvest and 22.414 l is the molar volume at STP (CPY-3, operators manual version 3.10; PP Systems). Net photosynthesis = CO_2 uptake in light, respiration = CO_2 efflux in dark, and gross photosynthesis = CO_2 uptake in light + CO_2 efflux in dark. H_2O flux ($\text{mmol m}^{-2} \text{s}^{-1}$) = $\Delta\text{H}_2\text{O} (V \times 1000)/(A \times 22.414 \times 1000 \times 60)$, where $\Delta\text{H}_2\text{O}$ is the difference of water vapour pressure between the air supply and the chamber pressure in millibars (CPY-3, operators manual version 3.10; PP Systems).

Climate and microclimate

Continuous measurements of PAR, UV-B and air temperature were recorded at the meteorological station 1 km from the experimental site, extracted from the ZERO database (Climate Basic) at the Zackenberg webpage (www.zackenberg.dk) and daily means are presented in

Fig. 2. The ambient UV-B radiation was monitored with an erythema-weighted UV-Biometer (Model 501; Solar light, Philadelphia, PA), incoming PAR radiation (LI-190SA; Li-Cor, Nebraska, NE) and air temperature in 2-m height (HMP 35D; Vaisala, Helsinki, Finland).

Microclimatic measurements were conducted in each plot. The leaf temperature was recorded at five different places in the canopy, representing both shaded and light exposed parts, with a non-contact IR thermometer (Raynger MX2; Raytek, Berlin, Germany) just before the canopy chamber was attached. Surface volumetric soil moisture content ($\text{m}^3 \text{m}^{-3}$) in 0–6 cm depth (Theta-probe type ML2x; Delta-T Devices Ltd., Cambridge, UK) and soil temperature in 5 and 10 cm depth (multi-thermometer) were measured at two different places outside the chamber base just after the detachment of the canopy chamber. PAR was recorded during the measurements by a sensor in the canopy chamber.

Leaves

The detached fresh leaves were digitally photographed immediately after the field measurements, and their areas were determined against a reference of known area by a pixel counting programme (S. Danbæk, Institute of Biology, University of Copenhagen). Subsequently, the leaves were dried at 80°C for 48–62 h, and their dry weights were determined. Total soluble flavonoids were

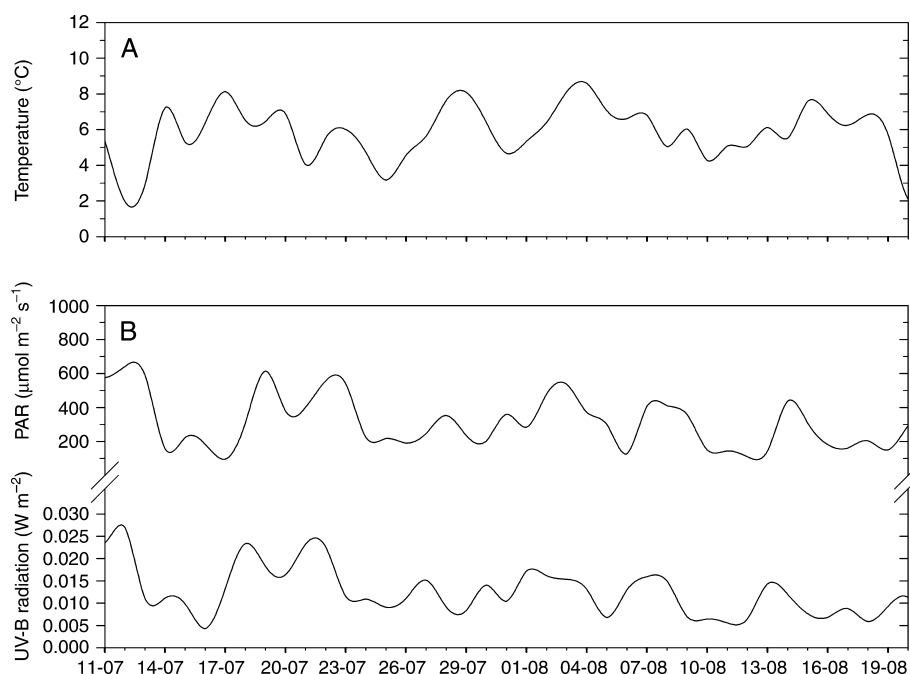


Fig. 2. (A) Air temperature in 2 m height. (B) PAR (upper) and daily erythema-weighted UV-B dose (lower). Twenty-four-hour means recorded at the meteorological station at Zackenberg from 11 July to 20 August 2002 are shown.

extracted by a three-step procedure: (1) heating (60°C) of pulverized leaves in 5 ml methanol for 3 min, (2) shaking 10 min after adding 5 ml HCl–H₂O–methanol (1:20:79), (3) diluting the supernatant by 10 in HCl–H₂O–methanol (1:20:179) after centrifugation at 1200 g for 10 min (Caldwell 1968). After additional 10-min shaking, the UV absorption was measured with a spectrophotometer (U-2010; Hitachi, Tokyo, Japan) in the interval between 280 and 315 nm by 0.2 nm increments. The area below the absorption curve was calculated as the sum of all absorptions in the scanned range. Carbon and nitrogen concentrations were determined on a Leco Truspec CN elemental determinator (Leco Corporation, St Joseph, MI).

Statistical analysis

Statistical analyses were conducted using the general linear model (GLM) procedure (SAS Institute Inc. 1999, version 8.02). Levene's test was used to test for homogeneity of variance and appropriate transformations were applied in cases with heteroscedasticity. Treatment effects on fluorescence variables were tested across all measurements during the season by one-way ANOVA. Gas exchange and microclimatic data were tested with a repeated measurement ANOVA, with treatment as factor and day repeated within each plot. This was to take into account that the same experimental unit (the plot) was measured several times during the season. In cases of significant treatment effects, these analyses were followed by tests of treatment differences using Tukey's test. All values presented here are non-transformed. Differences are considered at the $P < 0.05$ level. To exclude the possible interaction between treatment and microclimate on the gas exchange variables, Pearson's correlations between gas exchange variables and microclimatic parameters were calculated. If the correlations were significant ($P < 0.05$), then parameters were included in the ANOVA as covariates.

Results

Chl a fluorescence

The derived quantum efficiencies were slightly lower throughout the season for plants in ambient UV-B compared with plants in reduced UV-B. The $TR_o/ABS = F_v/F_M$ (efficiency by which an absorbed photon will be trapped by the PSII RC, with the resultant reduction of Q_A) was significantly decreased, where the ET_o/TR_o (efficiency by which an electron residing on Q_A^- will enter the intersystem electron transport chain) was only marginally decreased, but not significantly, in ambient UV-B. In combination, this led to a slight, but significant,

decrease in ET_o/ABS (quantum yield for electron transport) (Fig. 3, Table 1).

The derived specific fluxes per active PSII RC were almost all affected by treatment. Both the ABS/RC (absorption flux of photons per active RC) and the TR_o/RC (maximum trapping rate by which an exciton is trapped per active PSII RC) were significantly higher in ambient UV-B. In spite of this, ET_o/RC (electron transport to intersystem chain per active PSII RC) did not differ because of the significantly higher DI_o/RC (effective dissipated flux of untrapped excitons per active PSII RC) in ambient UV-B (Fig. 3, Table 1). The estimated number of active PSII RC per leaf CS using F_M , RC/CS_M , was significantly decreased in ambient UV-B (Fig. 3, Table 1).

The derived phenomenological fluxes per excited leaf sample CS, estimated from F_M , were reduced or unaffected in ambient UV-B across all measurements (Fig. 3, Table 1) except for late August (data not shown). The ABS/CS_M (photons absorbed, at F_M , by antenna molecules associated with all PSII RCs per leaf CS) was decreased in ambient UV-B, although not significantly (Fig. 3, Table 1) because of the late August increase for plants in ambient UV-B only (data not shown). The combination of significantly decreased TR_o/CS_M (trapping rate of excitons that will lead to Q_A reduction per leaf CS) and increased DI_o/CS_M (effective dissipated rate of untrapped excitons per leaf CS) led to a significant reduction in ET_o/CS_M (electron transport rate per leaf CS) in ambient UV-B. This pattern was seen throughout the season.

The overall performance of PSII was assessed by the performance indexes (both based on same quantum efficiencies) and both the PI_{Abs} (related to equal absorption) and PI_{CSM} (related to the per CS level by the

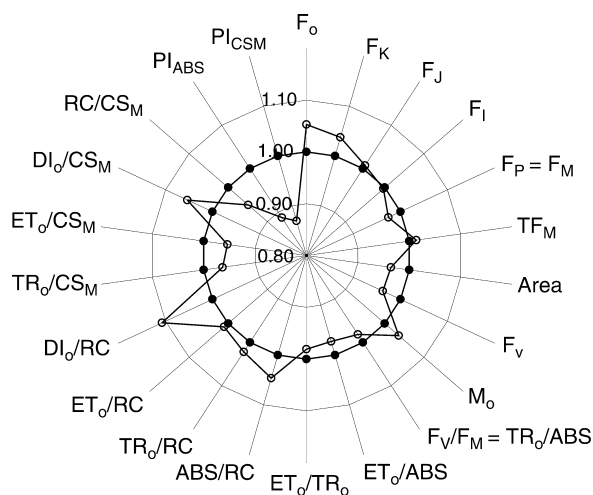


Fig. 3. Radar plot of fluorescence parameters. Mean values from Table 1 are indexed with reduced UV-B as reference level. Reduced UV-B is depicted with closed circles and ambient UV-B as open circles.

Table 1. Fluorescence variables. The numbers are seasonal means \pm SE tested by ANOVA with a general linear model followed by Tukey's test. Significant effects of treatment are shown by different letters. The resulting values of individual *F*-test and probability of correct null hypothesis are given. All data are extracted from the fast fluorescence transient O-J-I-P, according to Strasser et al. (2004). ABS, absorption energy flux; CS, excited cross-section of leaf sample; DI, dissipation energy flux at the level of the antenna chlorophylls; ET, flux of electrons from Q_A^- into the intersystem electron transport chain; φ_{PO} , maximal quantum yield of primary photochemistry; ψ_o , efficiency by which a trapped exciton, having triggered the reduction of Q_A to Q_A^- , can move an electron further than Q_A^- into the electron transport chain; RC, PSII reaction centre; RC/CS, concentration of reaction centres per excited CS of leaf sample; TR, excitation energy flux trapped by an RC and utilized for the reduction of Q_A to Q_A^- ; PI, performance index. Note the distinction between measured and derived parameters. See Materials and Methods and Introduction sections for an introduction to JIP-test, Fig. 2 for the indexed values of fluorescence parameters depicted as radar plot and Fig. 1 for pipeline models outlining specific and phenomenological fluxes.

Variable	Reduced UV-B	Ambient UV-B	<i>F</i> value	Prob <i>F</i>
Measured parameters				
$F_o = F_{50\mu s}$	210.4 ^B \pm 2.2	221.5 ^A \pm 3.3	9.62	0.0020
$F_K = F_{300\mu s}$	292.3 ^B \pm 2.7	303.3 ^A \pm 3.6	6.96	0.0085
$F_J = F_{2ms}$	493.2 ^A \pm 4.2	496.8 ^A \pm 4.5	0.034	0.5588
$F_I = F_{30ms}$	642.4 ^A \pm 5.7	640.3 ^A \pm 5.8	0.07	0.7868
$F_P = F_M$	823.2 ^A \pm 7.9	811.7 ^A \pm 7.7	1.01	0.3161
T_{FM}	834.1 ^A \pm 14.5	845.6 ^A \pm 14.7	0.38	0.5376
Area	28 307 ^A \pm 502	27 302 ^A \pm 482	2.17	0.141
Derived parameters				
Selected J-I-P-parameters				
F_V	612.9 ^A \pm 7.3	590.0 ^B \pm 6.8	5.55	0.0187
F_V/F_M	0.7345 ^A \pm 0.0032	0.7211 ^B \pm 0.0036	8.85	0.030
$(dV/dt)_0 = M_o$	0.5533 ^B \pm 0.0068	0.5731 ^A \pm 0.0082	3.36	0.0671
Quantum efficiencies				
$\varphi_{PO} = TR_o/ABS$	0.7345 ^A \pm 0.0032	0.7211 ^B \pm 0.0037	8.84	0.0030
$\varphi_{EO} = ET_o/ABS$	0.3915 ^A \pm 0.0032	0.3808 ^B \pm 0.0033	5.07	0.0246
$\psi_o = ET_o/TR_o$	0.5303 ^A \pm 0.0031	0.5201 ^A \pm 0.0034	0.39	0.5340
Specific fluxes				
ABS/RC	1.6230 ^B \pm 0.0195	1.698 ^A \pm 0.0242	6.79	0.0093
TR_o/RC	1.1637 ^B \pm 0.0073	1.1896 ^A \pm 0.0093	5.93	0.0150
ET_o/RC	0.6104 ^A \pm 0.0037	0.6165 ^A \pm 0.0039	2.02	0.1553
DI_o/RC	0.4593 ^B \pm 0.0142	0.5086 ^A \pm 0.0173	5.65	0.0176
Phenomenological fluxes				
$ABS/CS_M = F_M$	823.2 ^A \pm 7.9	811.7 ^A \pm 7.7	1.01	0.3161
TR_o/CS_M	612.8 ^A \pm 7.2	590.2 ^B \pm 6.8	5.55	0.0187
ET_o/CS_M	330.0 ^A \pm 4.8	314.8 ^B \pm 4.5	4.94	0.0264
DI_o/CS_M	210.3 ^B \pm 2.2	221.5 ^A \pm 3.4	9.62	0.0020
Density of PSII reaction centres				
RC/CS_M	542.9 ^A \pm 7.6	514.9 ^B \pm 7.4	7.45	0.0065
Performance indexes				
PI_{ABS}	2.48 ^A \pm 0.06	2.20 ^B \pm 0.05	9.51	0.0021
PI_{CSM}	2171 ^A \pm 61	1888 ^B \pm 54	12.03	0.0005

approximated absorption) showed same significantly decreased pattern during season in ambient UV-B (Fig. 3, Table 1).

The seasonal fluctuations of almost all above parameters showed a steep increase until late July and then parameters were stabilized at a plateau until mid-August and hereafter decreased throughout the season (data not shown).

Gas exchange

The canopy net photosynthesis showed an almost constant significant difference between reduced UV-B

and ambient UV-B plots from the beginning of the season until mid-August (Fig. 4A). The mean seasonal net photosynthesis was significantly decreased in ambient UV-B, also with PAR or leaf temperature as covariates, being 77% of P_n at reduced UV-B (Table 2). The seasonal fluctuations of net photosynthesis for both treatments follow, to some extent, the variation in PAR. Net photosynthesis was at its maximum in late July, where after a plateau was maintained until the late August autumn decrease. No difference between treatments was observed in late August.

Respiration showed a declining trend through season for both treatments (Fig. 4B). The mean seasonal

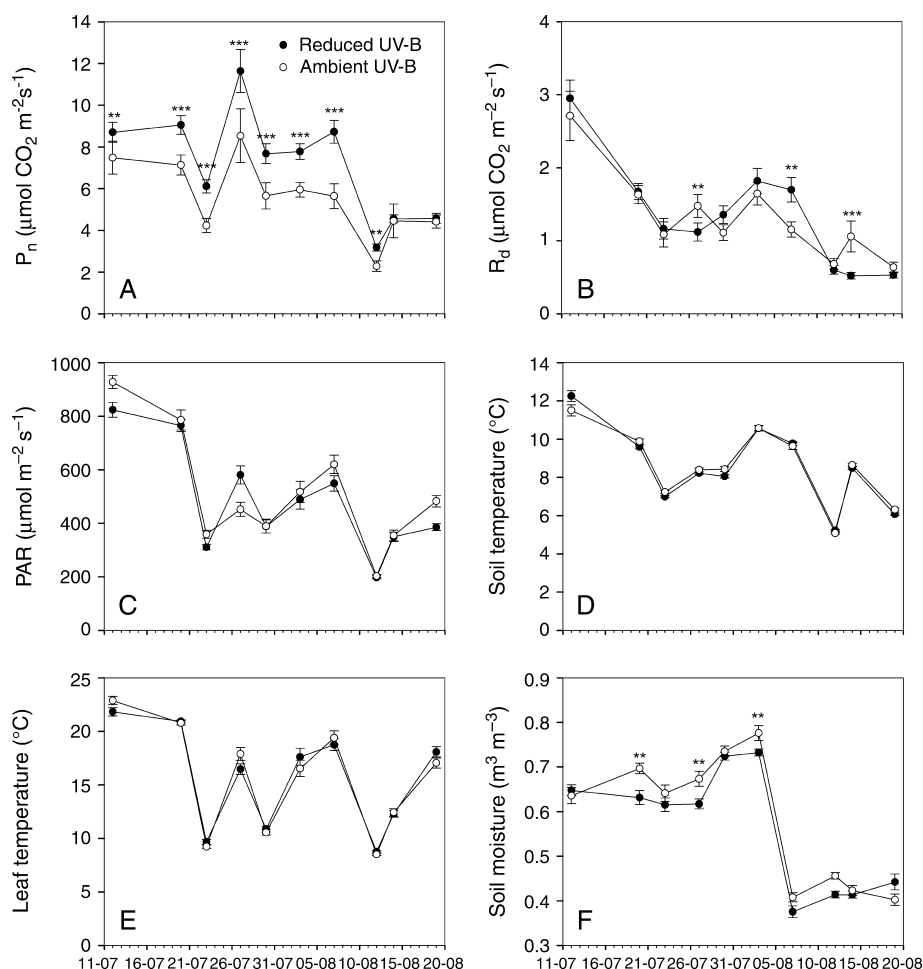


Fig. 4. (A) Net photosynthesis and (B) dark respiration on canopies of *Vaccinium uliginosum*. The daily means \pm SE during season are shown. Net photosynthesis is depicted as circles and respiration as squares. (C) PAR inside chamber during measurement, (D) soil temperature, (E) leaf temperature and (F) soil moisture in the *Vaccinium* canopies during season. Closed symbols represent reduced UV-B and open symbols represent ambient UV-B. *** $P < 0.0010$, ** $P < 0.0100$ and * $P < 0.1000$ tested by ANOVA with a general linear model followed specifying the repeated measurements (see Statistical analysis).

respiration in ambient UV-B plots was marginally lower than in reduced UV-B (except 27 July and 14 August) but only as a weak statistical tendency. Across all measurements through the season, there was a significant effect on respiration with soil temperature as covariate.

Microclimate

Leaf and soil temperatures were almost identical throughout the season, but soil water content differed significantly between treatments ($P < 0.0001$), being higher in ambient UV-B plot than in UV-B reduced plots (Fig. 4F).

Table 2. Gas exchange variables measured. The numbers are seasonal means \pm SE tested by ANOVA with a general linear model specifying the repeated measurements. PAR inside the chamber, leaf temperature or soil temperature were included as covariates (see Statistical analysis). The resulting value and probability of F -statistics is given.

Variable	Reduced UV-B	Ambient UV-B	Prob F
Net photosynthesis (P_n , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	7.20 ± 0.19	5.55 ± 0.22	0.0001
Dark respiration (R_d , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	1.34 ± 0.05	1.32 ± 0.06	0.0999
Gross photosynthesis (P_g , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	8.54 ± 0.22	6.88 ± 0.27	0.0407
Transpiration (T_r , $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	1.94 ± 0.05	1.94 ± 0.06	0.9262

In general, the fluctuations in temperatures followed the fluctuations in the PAR level (Fig. 4C–E), with soil temperature response being relatively dampened (Fig. 4f).

Growth and leaf chemistry

Growth parameters probably depends strongly on the initial values; hence, no difference in the measured mean leaf biomass, stem biomass and total aboveground biomass per plot as well as the estimated leaf area, specific leaf area (SLA) and leaf area index (LAI) was found between treatments (data not shown). No differences in leaf concentrations of carbon, nitrogen and C/N ratio were observed between the treatments. The leaf content of total soluble UV-B-absorbing compounds was significantly higher in ambient UV-B plots (80 area units) compared with the UV-B reduced plots (64 area units) ($P < 0.045$).

Discussion

PSII performance

The JIP-test reveals information on different scales of the performance of the PSII photosynthetic machinery and has proven sensitive to detect stress (Clark et al. 2000, Krüger et al. 1997). It has been pointed out that the PI indexes, similar to CO_2 assimilation, are regulated by accumulation of effects on both the biophysical and the biochemical performance of the photosynthetic apparatus (van Heerden et al. 2003). However, only one such investigation has been carried out in UV-B-exclusion experiments (Albert et al. 2005), but it was not coupled to photosynthetic rates, despite the potential new insight. For *V. uliginosum*, the relations between quantum efficiencies, specific fluxes per active PSII RC and phenomenological fluxes per leaf CS closely followed the pattern reported for *S. arctica* from the nearby experimental site (Albert et al. 2005) and was clearly negatively affected by ambient UV-B at all levels. This is elaborated in detail below.

Vaccinium quantum efficiencies were reduced in ambient UV-B, clearly indicating a slightly lower potential to process light energy through PSII. Moving onto the specific fluxes per active PSII RCs, the absorption flux of photons per active RC, ABS/RC , was higher in ambient UV-B. Probably this reflects a larger average antenna size per PSII RC. As the antennae size is calculated average values as total absorbing Chl per total fully active (Q_A reducing) PSII RCs, and it can be suggested that the antennae size would be increased if RCs were converted into heat sinks or other regrouping of antennae from active RCs to inactive RCs occurred (Strasser et al. 1995, van Heerden et al. 2003). This lead

to a larger maximum trapping rate of excitons per active PSII RC, TR_0/RC , but because of the higher effective dissipation of untrapped excitons per active PSII RC, DI_0/RC , this summed up to the same level of electron transport per PSII RC, ET_0/RC , in both treatments. Therefore, it is reasonable that the integrated response of individual fluxes per active PSII RCs into the performance index, PI_{Abs} , confirms the lower performance at this level. The combination of equal electron transport per active PSII RC (ET_0/RC) in both treatments and the decreased number of active PSII RCs per leaf CS, RC/CS_M , clearly indicate a reduced potential of electron transport capacity per leaf CS in ambient UV-B. This is in accordance with the interpretation by Albert et al. (2005), linking this particular behaviour of parameters between levels in response to UV-B. Indeed, a reduction in phenomenological fluxes per leaf CS in ambient UV-B was found and resulted in a 5% decreased electron transport per leaf CS, ET_0/CS_M . On the other hand, if phenomenological fluxes were estimated using F_o , an unchanged ET_0/CS_0 were observed (data not shown). This point to care when estimating phenomenological fluxes because they are directly affected by the magnitude of the here chosen estimators, F_o or F_M , and also the quantum efficiencies. This may be seen as a weakness of the JIP-test but also stresses the assumption that estimators theoretically shall reflect the absorption energy flux per excited CS of leaf sample (ABS/CS) in arbitrary units in the dark-adapted state (Strasser et al. 2004), may not always be easy to apply. However, independent of chosen estimators, the dissipation of untrapped photons per leaf CS was consistently highest in ambient UV-B. These points to the particular importance of ambient UV-B impact on energy dissipation processes. Differences in response pattern in quantum efficiencies and phenomenological fluxes are integrated in the performance indexes [PI_{CS_M} (Table 1) and also PI_{CS_0} (not shown)]. Their reduction clearly indicates a reduced overall processing of light energy per leaf sample CS in ambient UV-B. Hence, these response patterns do support the consideration that PI indexes are sensitive and overall integrating parameters for PSII performance (Clark et al. 2000, Strasser et al. 2000).

High irradiance levels above photosynthetic capacity decrease PSII activity (Krause and Weiss 1991), whereas medium-to-low irradiance levels do not. Therefore, the differences of PAR levels between filtered treatments are considered. Because the ambient UV-B treatment exposed the plants to a higher proportion of PAR compared with UV-B reduced treatment, the plants in ambient UV-B treatment could be argued to be more susceptible to a decrease in PSII activity. Therefore, we cannot rule out a possible small negative effect of PAR on the decrease in PSII activity. However, the much higher proportional

change in UV-B level do strengthen that the impact on PSII activity is because of ambient UV-B.

Altogether, these findings clearly indicate a negatively affected PSII performance and lower potential of electron transport capability in the chloroplasts of the plants exposed to ambient UV-B.

Photosynthetic performance

The gas exchange measured in the canopy plots is supposed, predominately, to be affected by the above-ground plant parts only because of the elevated pressure in the chamber. The sparse graminoids and the bottom layer of moss are thought to have a minor effect on gas exchange, and therefore, the main part of the gas exchange is ascribed to the canopy of *Vaccinium* alone.

In addition to the difference in UV-B levels, the plants in the filtered controls received approximately 11% more PAR compared with plants in reduced UV-B. As argued below, PAR is not a confounding factor here. The UV-B effects were very significant also when taking PAR levels into consideration. The PAR and UV-B effects were separated, and the PAR effect excluded by including PAR, measured simultaneously with each single gas exchange recording, in the statistical analysis as covariate (see Statistical analysis section). Moreover, it could be argued that light unsaturated plants exposed to different PAR levels could translate into different photosynthetic rates, why PAR levels at the time of measurement could be of special importance. This was not the case as the variation in the PAR levels, which alternated random between treatments, did not translate into higher photosynthesis in controls even when PAR levels were highest here. Additionally, if plants are argued to be measured under light-saturated conditions, the effects PAR should be equivalent and as such ruled out adding further to the UV-B effect. Finally, we argue that even if the approximately 11% less PAR level in UV-B reduced plots compared with filtered controls potentially could result in adjustments of photosynthetic capacity, then no such responses were seen as the photosynthesis rates were significantly highest in reduced UV-B.

No other reports on photosynthesis rates in relation to UV-B in the high Arctic are available, but decreased rates have been reported from other ecosystems. Decreased net photosynthesis for plants exposed to the realistic supplemental UV-B irradiance is in agreement with several previous reports (Baker et al. 1997, Keiller and Holmes 2001, Keiller et al. 2003), showing inhibition of photosynthesis in the field. Effects on growth have also been reported from exclusion experiments (Gonzalez et al. 1998, Krizek et al. 1997). Other studies performed with supplemental UV-B (Allen et al. 1999) did not find

such effects. During the senescence period, the treatment differences disappeared. This is different from earlier findings of UV-B promoted senescence in beech (Zeuthen et al. 1997). Although difficulties in results comparisons because of different methodologies, supplemental UV-B vs UV-B exclusion, have been pointed to (Flint et al. 2003, Rousseaux et al. 2004), several mechanisms have been suggested to be responsible for UV-B-induced stress on photosynthesis. Effects on gene expression in field conditions have been shown (Keiller et al. 2003), while indoor supplementation studies have identified changes in stomatal conductance (Negash and Björn 1986, Teramura et al. 1983), Rubisco content, reductions in capacity for photosynthetic electron transport (J_{\max}) and maximum carboxylase activity of ribulose 1,5-bisphosphate carboxylase/oxygenase ($V_{c,\max}$) (Allen et al. 1997, Baker et al. 1997, He et al. 1993, Jordan et al. 1992) as possible limitations on photosynthesis. These reductions have been argued to be caused by unrealistic high UV-B irradiances (Allen et al. 1998). Some studies suggested that PSII is the primary target of UV-B damage, reducing PSII activity (Melis et al. 1992, Strid et al. 1990) and abundance of D1 (Jansen et al. 1996, Nogués and Baker 1995), while other studies have shown that photosynthetic inhibition can occur without any measurable effect on PSII (Allen et al. 1999, Middleton and Teramura 1993). Indeed, it may not always be realistic to expect a close correlation between net photosynthesis and loss of functional PSII complexes, especially if PSII complexes are in excess. Hence, Lee et al. (1999) demonstrated, in vivo on *Capsicum annuum*, a limitation of photosynthetic capacity to be present only after a 40% loss of functional PSII complexes.

In this study, the lower quantum efficiencies, phenomenological fluxes, performance indexes and number of active RCs in ambient UV-B clearly indicated that ambient UV-B reduces the potential of primary photochemistry. The parameters may be criticized for being potentials and not actual fluxes because they are investigated on predarkened leaves. Ideally, the analysis of fluorescence parameters has to be evaluated in relation to incoming PAR, leaf absorbance, etc., while further studies should include simultaneous measurements of gas exchange and fluorescence parameters during light exposure. With these precautions, the observed reduction in net photosynthesis is at least partly explained by decreased PSII performance, although effects on other parts of the photosynthetic machinery, e.g. direct effects on the Calvin cycle function, are indeed very possible, especially when comparing the relative differences in treatment effects between PSII function and photosynthesis. The parallel seasonal variation in PSII performance and net photosynthesis strengthen linking of the impact of increased PSII performance to increased net photosynthesis.

UV-B-absorbing compounds

Enhanced synthesis of flavonoids (Caldwell et al. 1998, Searles et al. 2001) and hydroxycinnamates (Burchard et al. 2000, Ruhland et al. 2005) is a well-known response to increased UV-B radiation, why UV-B-absorbing compounds were expected to be more abundant in ambient UV-B compared with UV-B-excluded plots. This was confirmed in this study. In addition, parallel results were found in a 6-year UV-B-exclusion study on the same species on a nearby site (Albert et al., unpublished data). In comparison, studies carried out with supplemental UV-B in the sub-Arctic on *V. uliginosum* found a tendency to an increase in UV-B-absorbing compounds in response to supplemental UV-B (Phoenix et al. 2000, Semerdjieva et al. 2003a), but at the same time, a significant depletion in wall-bound pigments was found (Semerdjieva et al. 2003a). In contrast, no significant change in whole leaf extracts of UV-B-absorbing compounds (including cellular and epicuticular extracts) in sub-Arctic *V. uliginosum* was found in a 3-year UV-B-exclusion study (Phoenix et al. 2002). Together these findings clearly indicate differences in the performance of this species in response to UV-B. It has been suggested that the vulnerability to UV-B irradiance may be exacerbated in simple canopy systems in arctic vegetation (Phoenix et al. 2000). Moreover, sub-Arctic *V. uliginosum* may receive more UV-B irradiance than other dwarf shrubs as it is a strong competitor for light as it orients most leaves horizontally and has a very thin cuticle with no wax sculpturing and a low number of trichomes (Semerdjieva et al. 2003b). In comparison, high arctic *V. uliginosum* are even more exposed, and therefore, the differences could tentatively be interpreted as related to the UV-B doses in general or to differences between Arctic regions.

Conclusions

This study clearly demonstrated an almost immediate decrease in net photosynthesis in ambient UV-B compared with reduced UV-B. In addition, recordings of fluorescence induction curves and calculations of JIP-test parameters clearly demonstrated negative impact on PSII performance, i.e. F_V/F_M and performance indexes in ambient UV-B. In combination with the finding of a high amount of UV-B-absorbing compounds, this could be interpreted that the plants to some degree are protected against UV-B and that underlying tissues are only slightly affected by ambient UV-B. However, the lower photosynthesis clearly demonstrated that this was not the case in the present study. It must therefore be stressed that synthesis of parameters on different scales is needed when evalu-

ating the effects of UV-B. It should be noted that the 60% reduction of the UV-B irradiance used in this study implies a higher relative change in the UV-B load than many of the supplemental experiments do. Finally, the synthesis across scales within this study and the finding of the 24% increase in net photosynthesis when reducing the UV-B irradiance in particular clearly indicate that despite a clear UV-B avoidance response in canopies of *V. uliginosum*, this species is affected negatively by ambient UV-B levels.

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References

- Albert KR, Mikkelsen TN, Ro-Poulsen H (2005) Effects of ambient versus reduced UV-B radiation on high arctic *Salix arctica* assessed by measurements and calculations of chlorophyll-a fluorescence parameters from fluorescence transients. *Physiol Plant* 124: 208–226
- Alenius CM, Vogelmann TC, Bornman J (1995) A three dimensional representation of the relationship between penetration of UV-screening pigments in leaves of *Brassica napus*. *New Phytol* 131: 297–302
- Allen DJ, McKee IF, Farage PK, Baker NR (1997) Analysis of the limitation to CO₂ assimilation on the exposure of leaves of two *Brassica napus* cultivars to UV-B. *Plant Cell Environ* 20: 633–640
- Allen DJ, Nougues S, Baker NR (1998) Ozone depletion and increased UV-B radiation: is there a real threat to photosynthesis? *J Exp Bot* 49: 1775–1788
- Allen DJ, Nougues S, Morison JIL, Greenslade PD, Mcleod AR, Baker NR (1999) A thirty percent increase in UV-B has no impact on photosynthesis in well-watered and droughted pea plants in the field. *Global Chang Biol* 5: 235–244
- Baker NR, Nougues S, Allen DJ (1997) Photosynthesis and photoinhibition. In: Lumsden PJ (ed) *Plants and UV-B Responses to Environmental Change*, Society for experimental biology, Seminar Series 64. Cambridge University Press, Cambridge, UK, pp 95–112
- Björn LO, Callaghan TV, Gehrke C, Gwynn-Jones D, Lee JA, Johanson U, Sonesson M, Buck ND (1999) Effects of ozone

- depletion and increased ultraviolet-B radiation on northern vegetation. *Polar Res* 18: 331–337
- Bredahl L, Ro-Poulsen H, Mikkelsen TN (2004) Reduction of the ambient UV-B radiation in the high Arctic increases F_v/F_m in *Salix arctica* and *Vaccinium uliginosum* and reduces stomatal conductance and internal CO_2 concentration in *S. arctica*. *Arct Antarct Alp Res* 36: 364–369
- Burchard P, Bilger W, Weissenböck G (2000) Contribution of hydroxycinnamates and flavonoids to epidermal shielding of UV-A and UV-B radiation in developing rye primary leaves as assessed by ultraviolet-induced chlorophyll fluorescence measurements. *Plant Cell Environ* 23: 1373–1380
- Caldwell MM (1968) Solar ultraviolet radiation as an ecological factor for alpine plants. *Ecol Monogr* 39: 243–268
- Caldwell MM, Flint SD (1994) Stratospheric ozone reduction, solar UV-B radiation and terrestrial ecosystems. *Clim Change* 28: 375–394
- Caldwell MM, Björn LO, Bornman JF, Flint SD, Kulandaivelu G, Teramura AH, Tevini M (1998) Effects of increased solar ultraviolet radiation on terrestrial ecosystems. *J Photochem Photobiol B Biol* 46: 40–52
- Callaghan TV, Jonasson S (1995) Arctic terrestrial ecosystems and environmental change. *Philos Trans R Soc Lond A* 352: 259–276
- Clark AJ, Landolt W, Bucher JB, Strasser RJ (2000) How wind affects the photosynthetic performance of trees: quantified with chlorophyll-a fluorescence and open-top chambers. *Photosynthetica* 38: 349–360
- Cybulski WJ, Peterjohn WT (1999) Effects of ambient UV-B radiation on the aboveground biomass of seven temperate-zone plant species. *Plant Eco* 145: 175–181
- Day TA, Ruhland CT, Xiong FS (2001) Influence of solar ultraviolet-B radiation on Antarctic terrestrial plants: results from a 4-year field study. *J Photochem Photobiol B Biol* 62: 78–87
- Flint SD, Ryel RJ, Caldwell MM (2003) Ecosystem UV-B experiments in terrestrial communities: a review of recent findings and methodologies. *Agri For Meteorol* 120: 177–189
- Gehrke C, Johanson U, Björn LO, Callaghan TV, Lee JA (1996) Effects of enhanced ultraviolet-B radiation on terrestrial sub-arctic ecosystems and implications for interactions with increased atmospheric CO_2 . *Ecol Bull* 45: 192–203
- Gonzalez R, Mepsted E, Wellburn AR, Paul ND (1998) Non-photosynthetic mechanisms of growth reduction in pea (*Pisum sativum* L.) exposed to UV-B radiation. *Plant Cell Environ* 21: 23–32
- He J, Huang L-K, Chow WS, Whitecross MI, Anderson JM (1993) Effects of supplementary ultraviolet-B radiation on rice and pea plants. *Aust J Plant Physiol* 20: 129–142
- Jansen MAK, Gaba V, Greenberg BM, Mattoo AK, Edelman M (1996) Low threshold levels of ultraviolet-B in a background of photosynthetically active radiation trigger rapid degradation of the D2 protein of photosystem II. *Plant J* 9: 693–696
- Johanson U, Gehrke C, Björn LO, Callaghan TV (1995) The effects of enhanced UV-B radiation on the growth of dwarf scrubs in a subarctic heathland. *Func Ecol* 9: 713–719
- Jordan BR, He J, Chow WS, Anderson JM (1992) Changes in mRNA levels and polypeptide subunits of ribulose 1,5-bisphosphate carboxylase in response to supplemental UV-B radiation. *Plant Cell Environ* 15: 91–98
- Keiller DR, Holmes MG (2001) Effects of long term exposure to elevated UV-B radiation on the photosynthetic performance of five broad leaved tree species. *Photosynth Res* 67: 229–240
- Keiller DR, Mackerness SA-H, Holmes MG (2003) The action of a range of supplementary ultraviolet (UV) wavelengths on photosynthesis in *Brassica napus* L. in the natural environment: effects on PSII, CO_2 assimilation and level of chloroplast proteins. *Photosynth Res* 75: 139–150
- Krause GH, Weiss E (1991) Chlorophyll fluorescence and photosynthesis: the basis. *Annu Rev Plant Physiol Plant Mol Biol* 42: 313–349
- Krizek DT, Mirecki RM, Britz SJ (1997) Inhibitory effects of ambient levels of solar UV-A and UV-B radiation on growth of cucumber. *Physiol Plant* 100: 886–893
- Krüger GHT, Tsmilli-Michael M, Strasser RJ (1997) Light stress provokes plastic and elastic modifications in structure and function of photosystem II in camelia leaves. *Physiol Plant* 101: 265–277
- Lazár D (2006) The polyphasic chlorophyll a fluorescence rise measured under high intensity of exciting light. *Func Plant Biol* 33: 9–30
- Lee H-Y, Chow WS, Hong Y-N (1999) Photoinactivation of photosystem II in leaves of *Capsicum annuum*. *Physiol Plant* 105: 377–384
- Madronich S, McKencie RL, Björn LO, Caldwell MM (1998) Changes in biologically ultraviolet radiation reaching the Earth's surface. *J Photochem Photobiol B Biol* 46: 5–19
- Melis A, Nemson JA, Harrison MA (1992) Damage to functional components and partial degradation of photosystem II reaction centre proteins upon chloroplast exposure to ultraviolet-B radiation. *Biochim Biophys Acta* 1109: 312–320
- Middleton EM, Teramura AH (1993) The protective role of flavonol glycosides and carotenoids in protecting soybean from UV-B damage. *Plant Physiol* 103: 741–752
- Negash L, Björn LO (1986) Stomatal closure by ultraviolet radiation. *Physiol Plant* 66: 360–364
- Nogués S, Baker NR (1995) Evaluation of the role of damage to photosystem II in the inhibition of CO_2 assimilation in pea plants on exposure to UV-B radiation. *Plant Cell Environ* 18: 781–787
- Paul N (2001) Plant responses to UV-B: time to look beyond stratospheric ozone depletion? *New Phytol* 150: 1–8

- Phoenix GK, Gwynn-Jones D, Lee JA, Callaghan TV (2000) The impacts of UV-B radiation on the regeneration of a sub-arctic heath community. *Plant Ecol* 146: 67–75
- Phoenix GK, Gwynn-Jones D, Lee JA, Callaghan TV (2002) Ecological importance of ambient solar ultraviolet radiation to a sub-arctic heath community. *Plant Ecol* 165: 263–273
- Rasch M, Canning K (2003) Zackenberg Ecological Research Operations, 8th Annual Report. Danish Polar Center, Ministry of Science, Technology and Innovation, Copenhagen, Denmark, 80 pp
- Rinnan R, Keinänen MM, Kasurinen A, Asikainen J, Kekki TK, Holopainen T, Ro-Poulsen H, Mikkelsen TN, Michelsen A (2005) Ambient UV radiation in the Arctic reduces root biomass and alters microbial community composition but has no effects on microbial biomass. *Global Chang Biol* 11: 564–574
- Robson TM, Pancotto VA, Flint SD, Balleré CL, Sala OL, Scopel AL, Caldwell MM (2003) Six years of solar UV-B manipulations affect growth of Sphagnum and vascular plants in a Tierra del Fuego peatland. *New Phytol* 160: 379–389
- Rousseaux MC, Balleré CL, Giordano CV, Scopel AL, Zima AP, Szwarcberg-Bracchitta M, Searles PS, Caldwell MM, Diaz SB (1999) Ozone depletion and UV-B radiation: impact on plant DNA damage in southern America. *Proc Nat Acad Sci U S A* 96: 15310–15315
- Rousseaux MC, Flint SD, Searles PS, Caldwell MM (2004) Plant responses to current solar UV-B radiation and to supplemented solar ultraviolet-B radiation simulating ozone depletion: an experimental comparison. *J Photochem Photobiol B Biol* 80: 224–230
- Rozema J, Van de Staaij J, Björn LO, Caldwell MM (1997) UV-B as an environmental factor in plant life: stress and regulation. *Trends Ecol Evol* 12: 22–28
- Ruhland CT, Xiong FS, Dennis Clark W, Day TA (2005) The influence of ultraviolet radiation on growth, hydroxycinnamic acids and flavonoids of *Deschampsia antarctica* during springtime ozone depletion in Antarctica. *J Photochem Photobiol B Biol* 81: 1086–1093
- SAS Institute Inc. (1999) SAS/STAT Users guide, Version 8. Cary, NC
- Searles PS, Flint SD, Caldwell MM (2001) A meta-analysis of plant studies simulating stratospheric ozone depletion. *Oecologia* 127: 1–10
- Semerdjieva SI, Sheffield E, Phoenix GK, Gwynn-Jones D, Callaghan TV, Johnson GN (2003a) Contrasting strategies for UV-B screening in sub-Arctic dwarf shrubs. *Plant Cell Environ* 26: 957–964
- Semerdjieva SI, Phoenix GH, Hares D, Gwynn-Jones D, Callaghan TV, Sheffield E (2003b) Surface morphology, leaf and cuticle thickness of four dwarf shrubs from a sub-Arctic heath following long-term exposure to enhanced levels of UV-B. *Physiol Plant* 117: 289–294
- Strasser RJ, Srivastava A, Govindjee (1995) Polyphasic chlorophyll-a fluorescence transients in plants and cyanobacteria. *J Photochem Photobiol B Biol* 61: 32–42
- Strasser RJ, Tsimilli-Micheal M, Srivastava A (2000) The fluorescence transient as a tool to characterise and screen photosynthetic samples. In: Yunus M, Pathre U, Mohanty P (eds) *Probing Photosynthesis: Mechanisms, Regulation and Adaptation*. Taylor & Francis, London, pp 445–483
- Strasser RJ, Tsimilli-Micheal M, Srivastava A (2004) Analysis of the chlorophyll-a fluorescence transient. In: Papageogiou GC, Govindjee (eds) *Advances in Photosynthesis and Respiration*. Springer, Berlin, Germany, pp 321–362
- Strid Å, Chow WS, Anderson JM (1990) Effects of supplementary ultraviolet-B radiation on photosynthesis of in *Pisum sativum*. *Biochim Biophys Acta* 1020: 260–268
- Teramura AH, Tevini M, Iwanzik W (1983) Effects of ultraviolet-B irradiance on plants during a mild winter stress. I. Effects of diurnal stomatal resistance. *Physiol Plant* 57: 175–180
- Tsimilli-Michael M, Strasser RJ (2001) Fingerprints of climate changes on the photosynthetic apparatus' behaviour, monitored by the JIP-test. In: Walther G-R, Burga CA, Edwards PJ (eds) *"Fingerprints" of Climate Changes – Adapted Behaviour and Shifting Species Ranges*. Kluwer Academic/Plenum Publishers, New York, pp 229–247
- van de Staaij JWM, Ernst WHO, Hakvort HWJ, Rozema J (1995) Ultraviolet-B (280–320nm) absorbing pigments in the leaves of *Silene vulgaris*: their role in UV-B tolerance. *J Plant Physiol* 147: 75–80
- van Heerden PDR, Merope TM, Krüger GHJ, Strasser RJ (2003) Dark chilling effects on soybean genotypes during vegetative development: parallel studies of CO₂ assimilation, chlorophyll-a fluorescence kinetics O-J-I-P and nitrogen fixation. *Physiol Plant* 17: 476–491
- WMO (2003) Scientific Assessment of Ozone Depletion: 2002. Global Ozone Research and Monitoring Project. Report no. 47. World Meteorological Organization, Geneva, 498 pp
- Xiong FS, Day TH (2001) Effect of solar ultraviolet-B radiation during springtime ozone depletion on photosynthesis and biomass production of Antarctic vascular plants. *J Plant Physiol* 125: 738–751
- Zeuthen J, Mikkelsen TN, Paludan-Møller G, Ro-Poulsen H (1997) Effects of increased UV-B radiation and elevated levels of tropospheric ozone on physiological processes in European beech (*Fagus sylvatica*). *Physiol Plant* 100: 281–290

Paper VI

Solar Ultraviolet-B Radiation (UV-B) at Zackenberg: The Impact on Higher Plants and Soil Microbial Communities.

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Solar Ultraviolet-B Radiation at Zackenberg: The Impact on Higher Plants and Soil Microbial Communities

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SUMMARY

Depletion of the ozone layer and the consequent increase in solar ultraviolet-B (UV-B) radiation may impact living conditions for arctic plants significantly. In order to evaluate how the prevailing UV-B fluxes affect the heath ecosystem at Zackenberg (74°30'N, 20°30'W) and other high-arctic regions, manipulation experiments with various set-ups have been performed.

Activation of plant defence mechanisms by production of UV-B-absorbing compounds was significant in ambient UV-B in comparison to a filter treatment reducing the UV-B radiation. Despite the UV-B screening response, ambient UV-B was demonstrated to decrease photosynthesis and shift carbon allocation from shoots to roots. Moreover, ambient UV-B increased plant stress with detrimental effects on electron processing in the photosynthetic apparatus. Plant responses did not lead to clear changes in the amount of fungal root symbionts (mycorrhiza) or in the biomass of microbes in the soil of the root zone. However, the composition of the soil microbial

community was different in the soils under ambient and reduced UV-radiation after three treatment years.

These results provide new insight into the negative impact of current UV-B fluxes on high-arctic vegetation. They supplement previous investigations from the Arctic focussing on other variables like growth and so on, which have reported no or minor plant responses to UV-B, and the presented synthesis clearly indicates that UV-B radiation is an important factor affecting plant life at high-arctic Zackenberg. However, long-time experiments are needed in order to see whether the observed changes are transient or whether they accumulate over years. Such experiments are especially important for valid determination of below-ground responses, which potentially lead to feedbacks on the ecosystem functioning.

I. INTRODUCTION

The ecosystem responses to ultraviolet-B (UV-B) radiation (280–315 nm; CIE, 1999) in the Arctic are a research area of growing interest (Callaghan *et al.*, 2004a, 2005), motivating investigations focused to identify the targets and the relative importance of UV-B alone and in interaction with other global change factors. The increase in UV-B radiation is the result of stratospheric ozone depletion (Webb, 1997; Madronich *et al.*, 1998). The ozone-destroying chemical reactions are caused by chlorine and bromide released from emitted chloro-fluorocarbons and halons (Farman *et al.*, 1985) and the very cold stratospheric temperatures (below -78°C). The process is highly temperature dependent, it is most pronounced during spring with cold stratospheric conditions and it also varies from year to year (Weatherhead *et al.*, 2005). Currently, the UV-B irradiance level in the arctic region is considered to be near its maximum, and the ozone column is estimated to recover towards the middle of the century, but the rate of ozone recovery is uncertain in the northern hemisphere (WMO, 2003). Increased cloudiness decreases the amount of UV-B radiation reaching the ground (Madronich *et al.*, 1998), but the predictions of future cloud cover and cloud types are uncertain (Weatherhead *et al.*, 2005). The UV-B fluxes at Zackenberg ($74^{\circ}30'\text{N}$, $20^{\circ}30'\text{W}$), where this study was performed, peak in late May and early June, and high doses still prevail during July and August (see Figure 1 in Rinnan *et al.*, 2005). This means that changes in snow-cover and length of the growing season may affect the UV-B exposure dose of the vegetation. Moreover, vegetation located in the snow-free patches will receive greatly increased UV-B dose because of irradiance reflected from the surrounding snow (Jokela *et al.*, 1993; Gröebner *et al.*, 2000).

High-arctic plants are “living on the edge” because they are growing on the limit of their distribution in an extreme environment with a short growing season, low temperatures and often nutrient limitation. Therefore, acclimation

is of special importance especially when the plants face environmental changes, such as increased UV-B radiation (Caldwell *et al.*, 1980; Robberecht *et al.*, 1980), which can cause additional stress under ambient conditions (Bredahl *et al.*, 2004; Albert *et al.*, 2005a, 2007a). In addition, because the vegetation in the Arctic is evolutionary adapted to low UV-B levels (Robberecht *et al.*, 1980; Caldwell and Flint, 1994), the potential impact on the vegetation is expected to be pronounced (Björn *et al.*, 1999; Paul, 2001). This leads to the hypothesis that arctic plants are negatively influenced by the current UV-B levels. Thus, if the present UV-radiation affects the vegetation significantly, then reduction of the irradiance load would improve the photosynthetic performance of the plants. Therefore, an experimental approach where ambient UV-irradiance is screened off by means of filters was chosen in this study.

The previous knowledge of polar ecosystem responses to UV-irradiance is the result of field experiments with various experimental approaches: UV-radiation has been elevated by various lamp setups (e.g., Johanson *et al.*, 1995a,b; Björn *et al.*, 1999; Gwynn-Jones *et al.*, 1997; Phoenix *et al.*, 2001 and others), transplants have been set up along latitudinal gradients (Lehrner *et al.*, 2001), or UV-radiation has been reduced by means of filters (Xiong and Day, 2001; Phoenix *et al.*, 2002; Robson *et al.*, 2003; Albert *et al.*, 2005a). In principle, the studies supplementing UV-B relate closely to scenarios with future increased UV-B levels, whereas experimental UV-reduction relates to the impacts of the current level of solar UV-radiation.

UV-exclusion experiments by means of filters are attractive in several ways. They are simple and do not require electrical power or any special technical maintenance, which is an advantage in remote areas. Further, differences in spectral ratios, which are a problem in the UV supplementation experiments (Caldwell and Flint, 1994), can be avoided. However, reduction of the UV-B irradiance by 60% or more implies a higher relative change in the UV-B load than is predicted to take place in nature. Anyhow, the clear advantage to emphasise here is that the interpretation of results from UV-exclusion experiments directly relates to the impact of current level of UV-radiation, and that the exposure includes the variability during the growing season and from year to year.

The prevalent view is that—although UV-radiation induces increased production of phenolics and berries and alters the below-ground processes (Gwynn-Jones *et al.*, 1997; Searles *et al.*, 2001a; Johnson *et al.*, 2002)—arctic plants are more or less tolerant to enhanced UV-B in the long term (Phoenix *et al.*, 2001; Callaghan *et al.*, 2004b; Rozema *et al.*, 2006). Moreover, as discussed by Phoenix *et al.* (2001) and Phoenix and Lee (2004), other climatic changes, such as increased CO₂, temperature or changes in precipitation, may further negate the detrimental effects of enhanced UV-B in the sub-arctic.

II. VEGETATION AND PLANT ECO-PHYSIOLOGICAL RESPONSES AT ZACKENBERG

The experiments at Zackenberg approached the effects of ozone depletion on ecosystems by comparing the responses to prevailing UV-B fluxes to responses to reduced UV-levels obtained by different filter arrangements, which covered plots of the ecosystem (see [Box 1](#) for an outline of the conducted

Box 1

Overview of UV-B experiments at Zackenberg

UV-exclusion experiments were initiated in 2001, and until 2003 intensive monitoring of the microclimate in the experimental plots was done. The vegetation composition was mapped, and the hypothesised response parameters, such as photosynthesis, plant stress, leaf content of UV-B-absorbing compounds, carbon, nitrogen and soil characteristics, were investigated. The project is unique in the High Arctic and will be continued in cooperation with the ZERO programme.

2001 Establishment of two permanent sites differing in inclination (“level” and “sloping” sites) with four treatments in four groups: Filter treatments with UV-AB-reducing Lexan, UV-B-reducing Mylar, UV-transparent Teflon and a treatment without filter being an open control ([Box Figure 1](#)). Two independent climatic stations continuously logged microclimate: Air and soil temperatures, and in one group soil humidity, under and outside the filters as well as air humidity, air temperature and irradiance of photosynthetically active radiation (PAR) and UV-B at each site. Weekly measures of chlorophyll *a* fluorescence induction curves were conducted on bog blueberry *Vaccinium uliginosum* and arctic willow *Salix arctica* and end season harvest of leaves enabled analysis of UV-B-absorbing compounds. Gas exchange was measured on *S. arctica*. Results were published by [Bredahl et al. \(2004\)](#).

2002 Investigations on the permanent experimental plots were continued as the previous year. Moreover, two new experiments with UV-B-reducing Mylar and UV-transparent Teflon lasting one growth season were conducted: (1) Maximum irradiance experiment, where the irradiation doses were homogenised by controlling leaf angle on *S. arctica*, and intensive measures of chlorophyll fluorescence were conducted; (2) Robust measurements of photosynthesis were achieved by chamber measurements of gas exchange on whole canopies of *V. uliginosum* supplemented by chlorophyll fluorescence measurements. Results were published by [Albert et al. \(2005a, in press\)](#).

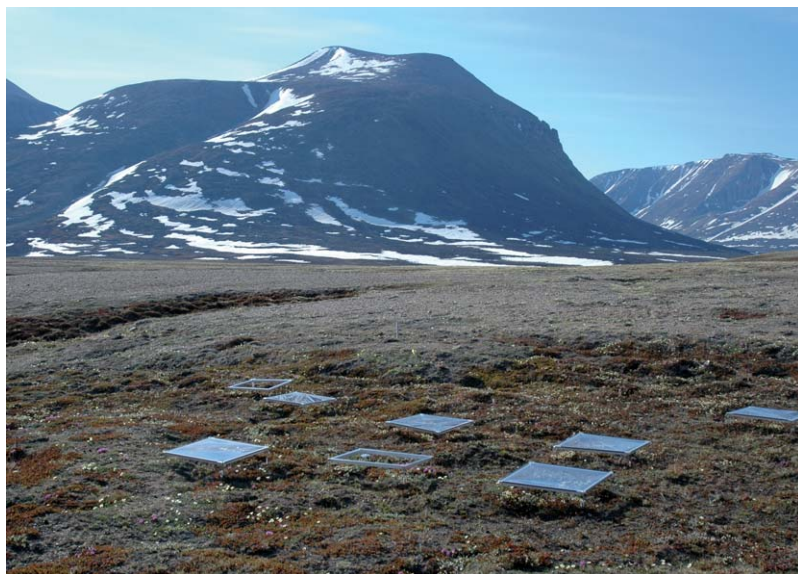
2003 Investigations on the permanent experimental plots were continued as in previous years, and in addition soil samples for root

biomass and microbial community analyses were taken. Continued experimentation with homogenised irradiation doses on *S. arctica* were performed on new plants shoots designed to achieve two different irradiance levels. Here, intensive measurements of both gas exchange and of chlorophyll fluorescence were conducted. Results were published by [Albert *et al.* \(2005b\)](#), [Rinnan *et al.* \(2005\)](#) and [Håkansson \(2006\)](#).

2004 No manipulations and no observations.

2005 Re-establishment of the permanent experimental plots and continuation of the measurements on *S. arctica* and *V. uliginosum* as done in previous years. Moreover, dwarf birch *Betula nana* was included on a new site with the same setup and measurement campaign. The experimentation with effects of homogenised irradiation doses on *S. arctica* was continued and measures of simultaneous chlorophyll *a* fluorescence and gas exchange were performed. Reflectance measures in the range 325–1250 nm were performed on all species, and leaves were sampled for analysis of secondary compounds, chlorophyll, C and N. Results presented in 11th Annual ZERO Report, 2005.

2006 Continuation of the measurement campaign on *V. uliginosum*, *S. arctica* and *B. nana*. Results presented in 12th Annual ZERO Report, 2006.



Box Figure 1 The experimental setup on a part of one of the permanent experimental sites (Site 2) comprising open control, filter control, UV-B-excluding Mylar filter and UV-AB-excluding Lexan.

Vegetation below filters is dominated by *Vaccinium uliginosum* and *Salix arctica*. In the background, the Zackenberg Mountain is seen. Photo: [K. Albert, 2002](#).

experiments and their responses). The four main treatments comprised open control plots without any filters, UV-transparent filter controls, filters reducing UV-B (280–315 nm) and filters reducing both UV-B and UV-A (315–400 nm). These treatments exposed the vegetation to *c.*100%, 91%, 39% and 17% of ambient UV-B radiation and 100%, 97%, 90% and 91% of the photosynthetically active radiation (PAR), respectively (Bredahl *et al.*, 2004). Filters also slightly changed the microclimate when compared to the open control. Three succeeding years of measurements during the growing season revealed that the filters increased the mean soil temperature up to 0.6°C, but this did not affect soil humidity. No significant mean air temperature difference was observed within the filter treatments or between the filter treatments and the open control. Between the filter treatments no microclimatic differences were found (K. Albert, unpublished).

From the range of UV-exclusion experiments conducted at Zackenberg, the emerging pattern is that there are significant plant-ecophysiological responses (Table 1). The chosen parameters, that is, photosynthesis and probing of plant stress, are generally expected to respond faster than many other important ecological processes, such as growth, phenology and species composition, which may respond to disturbances on a longer timescale (Callaghan *et al.*, 2004c). However, inter-species differences in plant performance characteristics are important since the traits are likely to influence competition and the resulting plant cover.

Measurements of chlorophyll *a* fluorescence induction curves led to calculation of the much reported parameter, maximal quantum yield (F_V/F_M), which closely relates to photosystem II (PSII) function and is often interpreted as a proxy for plant stress related to photosynthetic performance (Strasser *et al.*, 2004; see Box 2 for more information on photosynthesis and plant performance). Also the so-called performance index, PI (Strasser *et al.*, 2004) was derived from the fluorescence measurements. The PI integrates into one parameter the proportional responses of energy fluxes related to trapping and dissipation within the PSII and also to the energy transport behind PSII. Hereby, the PI expresses the overall effective energy processing through PSII and is believed to sum up the accumulative stress effects on PSII.

The UV-exclusion experiments were initiated in 2001 (see Box 1), and it was found that a reduction in the ambient UV-B level resulted in decreased content of UV-B-absorbing compounds and lower stress level indicated by increased F_V/F_M (Bredahl *et al.*, 2004) and PI in arctic willow *Salix arctica* and Bog blueberry *Vaccinium uliginosum ssp. microphyllum*. Moreover, the analysis of leaf level gas exchange revealed a decreased stomatal conductance and internal CO₂ concentration in *S. arctica* when ambient UV-B was reduced (Bredahl *et al.*, 2004). These findings were initially concluded to indicate the important impact of UV-B in the short term. However, the greatly varying leaf angle results in differences in UV-B doses for the

Table 1 Ecosystem responses to UV-B exclusion at Zackenberg

Response type	Parameters	Short-term effects			Long-term effects	
		<i>Salix</i>	<i>Vaccinium</i>	<i>Betula</i>	<i>Salix</i>	<i>Vaccinium</i>
Plant stress	Maximal photochemical efficiency (F_V/F_M)	↓ (~)	↓	↓	↓	↓
	Performance index (PI)	↓	↓	↓	↓	↓
Photosynthetic	Photosynthesis (P_n)	~	↓	#	#	#
	Respiration (R_d)	#	~	#	#	#
	Transpiration (Tr)	Δ	~	#	#	#
	Intercellular CO ₂ concentration (C_i)	↓	#	#	#	#
	Stomatal conductance (g_s)	↑	#	#	#	#
Growth	Leaf biomass	~	~	#	~	~
	Stem biomass	~	~	#	~	~
	Root biomass	#	#	#	#	↓
	Leaf area	~	~	#	~	~
	Specific leaf area	~	~	#	~	~
Leaf chemistry	UV-B-absorbing compounds	↑	↑	↑	↑	↑
	Carbon	~	~	~	~	~
	Nitrogen	~	~	~	~	~
Plant species composition	Chlorophyll	#	#	↓	#	#
	Cover	~	~	~	~	~
Mycorrhiza	Mycorrhizal colonisation	#	#	#	#	↑↓
	Root ergosterol concentration	#	#	#	#	~
Microbial biomass	Microbial biomass carbon	#	#	#	~	~
	Microbial biomass nitrogen	#	#	#	~	~
	Microbial biomass phosphorous	#	#	#	~	~
	Soil ergosterol concentration	#	#	#	~	~
	Phospholipid fatty acid (PLFA) biomarkers	#	#	#	Δ	Δ

Note: Ambient UV-B responses are compared to reduced UV-B after 1 and 3 years labelled short- and long term. Only significant changes are labelled with arrows. Signatures: No effect (~); Negative impact (↓); Positive impact (↑); Changed (Δ); Not investigated (#).

Box 2

Photosynthesis and plant performance

The photosynthetic processes can be separated into energy-producing (sources) and energy-consuming processes (sinks). Source processes are involved in the capture of light and the processing through the photosynthetic apparatus, resulting in available energy equivalents. Sink processes are the energy-demanding processes, primarily CO₂ assimilation in the Calvin cycle. The molecules of CO₂ diffuse into the leaf through stomatal openings into the stomatal cavities and from here further through the internal leaf cells until finally reaching the chloroplast, where the Calvin cycle takes place. Here, the CO₂ molecules assimilated are stored in energy-rich metabolites, that is, sugar, which is allocated to different plant parts for growth and maintenance. Under field conditions, the often used methods to probe both the characteristics and activity of the source and sink sides are to do measurements of chlorophyll *a* fluorescence and CO₂ and H₂O gas exchange. The sink processes are evaluated by parameters as, for example, net photosynthesis (P_n), stomatal conductance (g_s) and transpiration (Tr), while the source processes are evaluated by maximal quantum yield (F_v/F_m), performance indexes (PI), and a range of other parameters related both to the handling of light in the photosynthetic apparatus and partitioning of energy fluxes. The much reported parameter, maximal quantum yield (F_v/F_m), closely relates to photosystem II (PSII) function and is often interpreted as a proxy for plant stress. The PI integrates into one parameter the proportional responses of energy fluxes related to trapping and dissipation within the PSII and also to the energy transport behind PSII. Hereby, the PI expresses the overall effective energy processing through PSII and is believed to sum up the accumulative effects on PSII. Based on such measurements, the targets of environmental stressors can be both identified and quantified and the overall performance of the photosynthetic processes is assessed.

individual leaves, which probably confounded the effects. This hypothesis led to an experimental attempt to homogenise the UV-B dose received by the leaves by manipulative fixation of the plant leaves perpendicular to the Sun.

The second season (2002) included such a setup with fixation of leaves, and this led to clear-cut positive impacts on almost all measured and derived fluorescence parameters on *S. arctica*. The results on F_v/F_m and PI confirmed that ambient UV-B radiation is a significant plant stressor

(Albert *et al.*, 2005a). Also the proportions of energy fluxes per leaf cross section were quantified, and the dissipation of untrapped energy was highest under ambient UV-B, resulting in significantly lower flux of energy beyond the electron intersystem carriers (Albert *et al.*, 2005a). These responses are argued to be specifically due to the UV-B radiation (Albert *et al.*, 2005a), and they demonstrate a less effective energy processing in the photosynthetic machinery. For logistical reasons, no leaf level photosynthetic measurements were done on *S. arctica* that year, but measurements of photosynthesis and respiration were conducted on whole canopies of *V. uliginosum*. The *V. uliginosum* plants showed a decreased photosynthesis in parallel with decreased values of F_V/F_M and PI in ambient UV-B compared to the reduced UV-B treatment (Albert *et al.*, in press). This response was seen through most of the growth season, but in the senescence period in late August the treatment differences disappeared. These results clearly linked the decrease in net photosynthesis to the stress effects on the light-energy harvesting and processing machinery. Also the *V. uliginosum* plants had a higher level of UV-B-absorbing compounds in the leaves under ambient UV-B (Albert *et al.*, in press), but the possible protective screening by these compounds was obviously not sufficient to avoid negative effects on the photosynthetic machinery.

It has been argued by Searles *et al.*, (2001a) and others that changes in UV-B-absorbing compounds as such are not a good indicator of the degree of UV-B impact in plants, although the increase in UV-B-absorbing compounds in response to UV-B is the most consistent and frequent plant response (Searles *et al.*, 2001a). The pool here referred to as UV-B-absorbing compounds includes a range of secondary compounds, and moreover no distinction between wall bound and cellular compounds is generally made. These compounds have also other functions related to antioxidation (Bornman *et al.*, 1998; Rozema *et al.*, 2002) and plant defence against herbivores (Harborne and Grayer, 1993). Hence, the UV-induced alterations in the amount and quality of the UV-B-absorbing compounds may have implications for both herbivory (Ballaré *et al.*, 1996; Rousseaux *et al.*, 2004a) and litter decomposition (Björn *et al.*, 1999), affecting nutrient cycling, although this has not yet been investigated at Zackenberg.

For *S. arctica* plants, physiological responses between male and female plants may differ (Jones *et al.*, 1999; Håkansson, 2006). In a short-term study in 2003 on *S. arctica* at Zackenberg, the sex actually interacted with treatment responses, and surprisingly no significant treatment differences were found on F_V/F_M , photosynthesis or content of UV-B-absorbing compounds. The response was in general ascribed to the particularly high content of UV-B-absorbing compounds, being more than 50% higher than previous years, leading to effective screening against UV-B radiation. If this explanation is correct, then it suggests that plants under some conditions actually are able to cope with

the negative impact of UV-B. Håkansson (2006) also investigated the effect of sudden filter removal in filter treatments during peak season. Although no treatment-specific responses *per se* were detected hereafter, the plants actually became more stressed when re-exposed to ambient UV-B. This was indicated by decreased F_v/F_m , whereas the cohort of plants still being treated with filters showed the opposite and decreased their stress level.

Throughout the 3-year period (2001–2003), it was consistently found that *S. arctica* and *V. uliginosum* leaves exposed to current UV-B fluxes had higher content of UV-B-absorbing compounds and were experiencing a higher stress level than when UV-B was reduced (K. Albert, unpublished).

An investigation on dwarf birch *Betula nana* during 2005 clearly demonstrated a similar plant stress release by UV-reduction as earlier observed in *S. arctica* and *V. uliginosum*. F_v/F_m and PI were significantly increased throughout the experimental period in July and the beginning of August in the treatments where large proportions of UV-B and UV-AB were excluded as compared to both filtered and open control (Albert *et al.*, 2006). The stress response was previously hypothesized to be restricted to periods with high irradiance (of both PAR and UV) (Albert *et al.*, 2005a), and this was tested by measurements throughout a day under clear sky conditions. As expected, a midday depression in both F_v/F_m and PI was seen in *B. nana* across treatments in parallel with irradiance doses, which were maximal when the Sun was in Zenith. Surprisingly, the level of the PIs in the UV-reduction treatments stayed higher during all times of the day. This demonstrates that the control plants, which were exposed to the ambient level of UV-radiation, appear to be permanently stressed and do not recover after exposure to the midday high irradiation event by finalising repair processes. This new finding points to the importance of negative impacts of ambient UV-radiation on the photosynthetic apparatus in *B. nana*, which may be rendered as a UV-sensitive plant species, at least in the short term.

III. COMPARISONS OF PLANT RESPONSES THROUGHOUT THE POLAR REGION

Although caution is needed when making generalisations of polar plant responses because of differences between Antarctic and arctic ecosystems (e.g., higher species diversity, more trophic interactions and lower UV-B fluxes in the Arctic) (Rozema *et al.*, 2005), our observations are in agreement with the UV-exclusion studies conducted in the Antarctic ecosystems showing that ambient UV-B can have significant impacts.

Responses from the UV-exclusion studies carried out on the Antarctic Peninsula and in sub-Antarctic Tierra del Fuego point to negative UV-B effects

on plant growth (Day *et al.*, 1999; Rousseaux *et al.*, 1999; Ruhland and Day, 2000; Ballaré *et al.*, 2001 and others) and increased phenolic production in most species (Day *et al.*, 2001; Searles *et al.*, 2001a,b). Further, DNA damage (Rousseaux *et al.*, 1999) has been observed particularly during the high spring-time UV-B fluxes (Xiong and Day, 2001; Ruhland *et al.*, 2005) and also in the longer term (Robson *et al.*, 2003). The negative impact on plant biomass production, as reported by Xiong and Day (2001), was not associated with reduced photosynthesis per leaf area, but rather with reduced photosynthesis per chlorophyll amount or leaf dry mass. Xiong and Day (2001) interpret this response as that under UV-B, the plants were denser and probably had thicker leaves with a higher amount of photosynthetic and UV-B-absorbing pigments per leaf area. On the other hand, the analysis of chlorophyll *a* fluorescence and photosynthetic light response curves demonstrated that photosynthesis was impaired in the upper cell layers, but this did not translate into changes in photosynthetic rates at the whole leaf level.

There are differences between the responses observed at high-arctic Zackenberg compared to high-arctic Svalbard and the sub-arctic Abisko in northern Sweden, where most UV-B supplementation studies have been conducted.

No effects of 7 years of UV-B supplementation were detected on plant cover, density, leaf weight, leaf area, reproductive parameters, leaf UV-B absorbance and content of total phenolics in plants on arctic Svalbard (Rozema *et al.*, 2006). The absence of responses to enhanced UV-B in Svalbard was discussed to indicate several aspects. First, the differences in UV-B levels posed in supplemental studies are less than in UV-B exclusion studies, where responses were argued to be more difficult to detect. Secondly, the tundra biome in Svalbard originates from latitudes with higher natural solar UV-B fluxes implying a possible higher tolerance to UV-B (Rozema *et al.*, 2006).

Based on long-term studies conducted in the area of Abisko, it has been concluded that the dwarf shrubs there seem tolerant to ambient UV-B (Phoenix *et al.*, 2001, 2002; Callaghan *et al.*, 2004b; Rozema *et al.*, 2006). In some instances, enhanced UV-B radiation reduced plant growth, modified plant—herbivore interactions (Gwynn-Jones *et al.*, 1997), slowed the rate of litter decomposition, altered microbial soil biomass (Johnson *et al.*, 2002) and reduced cyanobacterial nitrogen fixation (Solheim *et al.*, 2002), but did not change plant cover or DNA damage (Rozema *et al.*, 2005, and references herein).

The investigations in sub-arctic Abisko and high-arctic Svalbard have put more weight on traditional parameters such as various measures of growth, phenology and so on, while the Zackenberg research has had a more non-invasive approach by weighting photosynthetic and stress variables, which respond immediately to changes in radiation. To take advantage of both

approaches, work focused on linking variables across scales, that is, the photosynthetic response to other measures of growth, should be done. However, because of the difference in variables measured and also a different experimental approach (supplementing UV-B vs UV-B exclusion), direct comparisons are not always possible. In the [section V](#) below, these important issues are discussed further.

IV. BELOW-GROUND RESPONSES

While above-ground plant responses have received much attention over the years, possible effects of UV-B radiation on below-ground components of arctic ecosystems are less well understood. Although the presence of creeping tundra plants leads to higher UV penetration to the soil compared to the presence of more shading cushion plants, grasses and mosses ([Hughes et al., 2006](#)), UV-radiation mainly affects the soil communities indirectly via effects on plants.

Ambient UV-B radiation at Zackenberg reduced root biomass of *V. uliginosum* as determined by soil core sampling after 3 years of UV-B exclusion ([Rinnan et al., 2005](#)). The lower root biomass is well in agreement with the responses in the above-ground plant parts, as ambient UV-B also reduced photosynthesis and induced stress to the photosynthetic machinery ([Bredahl et al., 2004](#); [Albert et al., in press](#)) as discussed above. Reductions in below-ground plant components due to ambient UV-B levels have also been reported for southern high latitudes. For instance, root length production of *Carex* spp. at a fen in southern Argentina was significantly lower under near-ambient than under reduced UV-B radiation ([Zaller et al., 2002](#)). At Palmer Station, at the Antarctic Peninsula, near-ambient UV-B radiation reduced root biomass of the Antarctic hair grass *Deschampsia antarctica* by 34% compared to the plants under reduced UV-B radiation ([Ruhland et al., 2005](#)). However, in this case the above-ground biomass was reduced even more, which led to a higher root-to-shoot ratio under near-ambient UV-B ([Ruhland et al., 2005](#)).

Changes in plant photosynthesis and carbon allocation are likely to have an impact on mycorrhizal symbionts living in association with plant roots. At Zackenberg, the response of mycorrhizal fungi to UV-B manipulations was unclear at the level site (see [Box 1](#) for the details of experimental setup). The light microscopical analyses indicated that the roots of *V. uliginosum* were more colonised by ericoid-type mycorrhiza under reduced UV-B radiation, but at the sloping site, the response was nearly opposite ([Rinnan et al., 2005](#)). The only other report on effects of UV-B radiation on ericoid mycorrhiza that we are aware of states that 5 years of UV-B enhancement by fluorescent

lamp arrays simulating 15% ozone depletion at Abisko had no effects on mycorrhizal colonisation (Johnson, 2003).

Net primary production (Callaghan *et al.*, 2004c) often correlates with the soil microbial biomass (Wardle, 2002). At our sites at Zackenberg, microbial biomass determined by the fumigation–extraction technique was, indeed, significantly associated with the total root biomass per soil volume ($R^2 = 0.23$, $p < 0.01$). However, the UV-B manipulations had no statistically significant effects on the soil microbial biomass or concentrations of nitrogen and phosphorus in the biomass (Rinnan *et al.*, 2005). This is in contrast with the results from a sub-arctic heath at Abisko, where UV-B supplementation for 5 years resulted in lower soil microbial biomass carbon and higher microbial biomass nitrogen concentration (Johnson *et al.*, 2002). However, a similar UV-B supplementation as at Abisko had no effects on the soil microbial biomass in a mesotrophic sub-arctic mire in northern Finland (R. Rinnan, unpublished data).

Potential UV-B-induced changes in the chemical quality and quantity of the labile carbon substances exuded from plant roots (i.e., root exudates) could affect soil microbial community composition. In order to compare the composition of the microbial communities between the UV-B treatments in our experiments at Zackenberg, we extracted phospholipid fatty acids (PLFAs) from the soil, which are biomarkers specific to different bacteria and fungi (Zelles, 1999). The PLFA profiles were indeed different under ambient and reduced UV-B fluxes (Rinnan *et al.*, 2005), which indicates that ambient UV-B radiation in Greenland has indirect effects on the soil microbial communities. This finding is supported by results both from the sub-arctic (Johnson *et al.*, 2002; R. Rinnan, unpublished data) and from Antarctica (Avery *et al.*, 2003), which reported effects of UV-B radiation on the utilisation of different carbon sources by culturable soil bacterial community. As the fungal biomass in the soil from Zackenberg was not affected by UV-B radiation based on the quantity of fungal PLFA biomarkers and ergosterol concentration (Rinnan *et al.*, 2005), the community composition alterations appeared to occur within the bacterial community.

Relating microbial community composition to microbial-driven ecosystem processes such as decomposition and nutrient transformations in the soil is not straightforward. Therefore, it is not possible to extrapolate how UV-B radiation would affect ecosystem functioning based on the observed responses in microbial community composition. Further analyses of microbial community by molecular methods and targeted measurements of ecosystem processes, such as nitrogen transformations, could reveal whether a certain group of bacteria was especially affected. As the indirectly induced below-ground responses can first take place after a strong enough response has occurred in plants, a 3-year-long experiment may not be long enough to show the eventual responses.

V. METHODOLOGICAL CONSIDERATIONS OF THE EXPERIMENTAL APPROACHES

In many cases, the drivers of ecosystem responses can best be identified by an experimental approach. Ideally, this implies well-documented long-term multi-factorial manipulations and comprehensive effects investigations. This approach takes advantage of testing the actual impact of the hypothesised driver and their interactions on ecosystem processes. If the environmental perturbations are realistic and well conducted they not only identify key ecosystem responses but also reveal their strength and relative importance in time and space. The syntheses of such results are the starting point for generation of novel hypotheses, which may be tackled via new experiments and relevant ecosystem modelling.

Concerning the responses to UV-B radiation: Is it possible to extrapolate results from UV-B exclusion experiments to future scenarios of ozone depletion and UV-B radiation climate? This exercise demands a range of premises to be discussed of which the most important are outlined below.

The supplemental studies which are closely simulating future scenarios may be argued to be far more realistic. UV-B exclusions substantially change the total UV-B irradiation to a much higher degree (up to 60%) than supplemental UV-B studies simulating 15–30% enhancement of UV-B do. Hence, UV-exclusion may *per se* be expected to induce greater responses in a dose-dependent context. Also the qualitative spectral differences existing between methodologies may be of importance. This is clearly indicated from studies applying treatments that reduce UV-B (UV-B-absorbing filter), ambient (UV-transmitting filter) and supplemental UV-B (UV-transmitting filter + lamps) in parallel (Gabeřšcik *et al.*, 2002; Rousseaux *et al.*, 2004b). Here, a stepwise dose-dependent UV-B response is to be expected on affected parameters, if responses are linear. Further, since the initiation of biological responses also are closely related to the spectral composition of light this adds to complexity.

To approximate the biological effective differences, which may be mediated by such differences in spectral composition, biological spectral weighting functions (BSWS) has been used by Rousseaux *et al.* (2004b). Here, biologically effective UV-B doses were calculated according to widely accepted and much used BSWS in an experiment with reduced UV, ambient UV-B and supplemental UV-B (30%) in parallel. Depending on BSWS, the doses differed by 1.4 to 6.4 times by comparing ambient UV-B to supplemental UV-B, whereas UV-doses differed by 1.5–77 times when comparing reduced UV-B to ambient UV-B. From this, it was concluded that considerable care is needed when comparing studies using the two different methodologies (Rousseaux *et al.*, 2004b).

If plants do not have linear responses to realistic doses of UV-B, are responses then subject to any thresholds? Does this relate to whether responses

are equally detectable by either approach? This is an area of dispute, but the directions of responses of parameters, such as UV-B-absorbing compounds, stomatal density, chlorophyll, transpiration and photosynthesis (although depending on species), are the same as with increases in UV-B within those few studies comprising reduced, ambient and enhanced UV-B radiation in parallel (Gabeřšcik *et al.*, 2002; Rousseaux *et al.*, 2004b). In addition, most of these responses display stepwise or dose-dependent changes (Gabeřšcik *et al.*, 2002; Rousseaux *et al.*, 2004b), although they probably are not universal. Concerning the phenolics, which also function as UV-B-absorbing compounds, UV-B exposure response curves have shown that the production of several phenolics quantitatively are UV-B dose dependent (de la Rosa *et al.*, 2001), but complex contrasting responses have also been seen. An increase in UV-B-absorbing compounds were found in supplemental UV-B (Phoenix *et al.*, 2000; Semerdjieva *et al.*, 2003a, 2003b), but no changes occurred when UV-B was excluded (Phoenix *et al.*, 2002) on sub-arctic *V. uliginosum*. Moreover, along a wide range of UV-B doses no evidence of a possible threshold UV-B dose for UV-B responses has been found (González *et al.*, 1998; de la Rosa *et al.*, 2001). Together these findings provide support that our approach is scientifically acceptable.

The fact that filter treatment may induce important microclimatic differences has to be taken into account. Filters may potentially change temperatures and humidity in air and soil and of course exclude rainwater. At Zackenberg, the filters were shown to only change soil temperature significantly, but this increase did not change the soil humidity. This was probably due to the very low precipitation during 2001–2003 growth seasons (Sigsgaard *et al.*, 2006) and that the filter plots were placed in an angle allowing vegetation to benefit from events of precipitation due to surface runoff from above the filters. Since filter treatments elevated mean soil temperature by 0.6°C, the UV effects may be viewed as a combined effect (warming plus UV-B reduction) compared to warming (filter control) and open control (no filter and no warming).

A special issue concerning the photosynthetic response is that the filter differences in PAR transmission may lead to differences in canopy photosynthesis (Flint *et al.*, 2003). The degree of photosynthesis impact depends on leaf area index (LAI) being increased by increased LAI. According to Flint *et al.* (2003), with an LAI of 1, 5–10% differences in PAR results in 2–4% difference in photosynthesis. Compared to the open control, the transmitted PAR is 97% and 90% in the filtered control and UV-B-reducing treatment, respectively. Thus, between filter treatments, the resulting difference is *c.* 7% less PAR-irradiance in the UV-B-reducing treatments, respectively. Since we observed a stepwise higher photosynthesis in parallel with less PAR-irradiance, the effect may be of little importance here.

In summary, taking the premises above into consideration, we believe that the UV-B exclusion approach is very well suited to identify the impact on

ecophysiological processes of current UV-B fluxes, whereas a UV-B supplemental approach may be better suited when evaluating their consequences. Hence, we argue that it is possible to indicate the direction of future ecosystem responses, but it remains speculative to actually quantify the responses within a particular UV-B radiation scenario, primarily because we do not know the UV-B exposure response curves and response to differences in the spectral composition of light.

VI. CONCLUSIONS AND FUTURE DIRECTIONS

The range of significant responses seen in the UV-B exclusion experiments at Zackenberg clearly indicates that ambient UV-B is a plant stress factor in this area. This seems in contrast to the reported robustness towards supplemental UV-B for plants in sub-arctic Abisko and high-arctic Svalbard. However, the results from UV-B exclusion studies in the Antarctic region have demonstrated effects on plants, similar to the results from Zackenberg. There are differences in the chosen response variables, and the contrasting responses may be interpreted to be due to the climatic differences between the areas. Further, the extreme living conditions in the high-arctic Zackenberg and Antarctic region may to a larger degree amplify effects of the stress factors, leading to significant UV-B impacts here. Although the responses from Zackenberg provides new insight and supplements earlier work, more work dedicated to link variables across scales is needed to take full advantage of the earlier findings. Thus, only by making parallel UV-B supplementation and UV-B exclusion field experiments it is possible to exclude the methodological differences and validate the ecosystem responses. Furthermore, the experiments should be conducted over longer time periods and include more traditional parameters (e.g., shoot growth rate and biomass effects) in order to ease comparisons and to elucidate whether the observed changes are transient or whether they accumulate over years. Long-term experiments are especially important for valid determination of below-ground responses, which have the potential to pose great feedbacks on the ecosystem functioning.

If projections from climatic scenarios to future biological responses shall be made, the biological responses and their feedback must be detected in multi-factorial experiments closely resembling the climatic projections. If sufficient reliable biological response functions to climatic parameters can be established then ecosystem modelling shall be possible. Presently, we do not have sufficient knowledge of all responses of importance and their interactions. Concerning ozone layer depletion, a specific UV-B radiation scenario for Zackenberg is needed. What we can state is that ambient UV-B as a single factor affects plant life negatively at high-arctic Zackenberg, and that the methodology developed is very well suited for long-term monitoring.

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REFERENCES

- Albert, K.R., Mikkelsen, T.N. and Ro-Poulsen, H. (2005a) *Physiol. Plantarum* **124**, 208–226.
- Albert, K.R., Ro-Poulsen, H., Mikkelsen, T.N., Bredahl, L. and Haakansson, K.B. (2005b) *Phyton* **45**, 41–49.
- Albert, K.R., Arndal, M.F., Michelsen, A., Tamstorf, M.F., Ro-Poulsen, H. and Mikkelsen, T.N. (2006) In: *Zackenberg Ecological Research Operations, 11th Annual Report, 2005* (Ed. by M. Rasch and K. Caning), pp. 90–91. Danish Polar Center, Ministry of Science, Technology and Innovation, Copenhagen.
- Albert, K.R., Mikkelsen, T.N. and Ro-Poulsen, H. (in press) *Physiol. Plantarum* in press.
- Avery, L.M., Smith, R.I.L. and West, H.M. (2003) *Polar Biol.* **26**, 525–529.
- Ballaré, C.L., Scopel, A.L., Stapelton, A.E. and Yanovsky, M.J. (1996) *Plant Physiol.* **112**, 161–170.
- Ballaré, C.L., Rousseaux, M.C., Searles, P.S., Zaller, J.G., Giordano, C.V., Robson, T.M., Caldwell, M.M., Sala, O.E. and Scopel, A.L. (2001) *J. Photoch. Photobio. B* **62**, 67–77.
- Björn, L.O., Callaghan, T.V., Gehrke, C., Gwynn-Jones, D., Lee, J.A., Johanson, U., Sonesson, M. and Buck, N.D. (1999) *Polar Res.* **18**, 331–337.
- Bornman, J.F., Reuber, S., Cen, Y.-P. and Weissenböck, G. (1998) In: *Plant and UV-B. Responses to Environmental Change* (Ed. by P.J. Lumsden), pp. 157–170. Cambridge University Press, Cambridge.
- Bredahl, L., Ro-Poulsen, H. and Mikkelsen, T.N. (2004) *Arct. Antarct. Alp. Res.* **36**, 363–368.
- Caldwell, M.M. and Flint, S.D. (1994) *Climatic Change* **28**, 375–398.
- Caldwell, M.M., Robberecht, R. and Billings, W.D. (1980) *Ecology* **61**, 600–611.
- Callaghan, T.V., Björn, L.O., Chernov, Y., Chapin, T., Christensen, T.R., Huntley, B., Ims, R.A., Johansson, M., Jolly, D., Jonasson, S., Matveyeva, N., Panikov, N., et al. (2004a) *Ambio* **33**, 474–479.

- Callaghan, T.V., Björn, L.O., Chernov, Y., Chapin, T., Christensen, T.R., Huntley, B., Ims, R.A., Johansson, M., Jolly, D., Jonasson, S., Matveyeva, N., Panikov, N., *et al.* (2004b) *Ambio* **33**, 418–435.
- Callaghan, T.V., Björn, L.O., Chernov, Y., Chapin, T., Christensen, T.R., Huntley, B., Ims, R.A., Johansson, M., Jolly, D., Jonasson, S., Matveyeva, N., Panikov, N., *et al.* (2004c) *Ambio* **33**, 398–403.
- Callaghan, T.V., Björn, L.O., Chapin, T., Chernov, Y., Christensen, T.R., Huntley, B., Ims, R.A., Johansson, M., Riedlinger, Jolly, D., Jonasson, S., Matveyeva, N., Oechel, W., *et al.* (2005) In: *ACIA, Arctic Climate Impact Assessment* (Ed. by C. Symon, L. Arris and B. Heal), pp. 243–352. Cambridge University Press, New York.
- CIE (1999) *134/1:TC 6–26 report: Standardization of the terms UV-A1, UV-A2 and UVB*. Commission Internationale de l'Eclairage (CIE). Collection in Photobiology and Photochemistry, Vienna, Austria CIE 134–1999 ISBN 3 900 734 94 1.
- Day, T.A., Ruhland, C.T., Grobe, C.W. and Xiong, F. (1999) *Oecologia* **119**(1), 24–35.
- Day, T.A., Ruhland, C.T. and Xiong, F.S. (2001) *J. Photoch. Photobio. B* **62**, 78–87.
- de la Rosa, T.M., Julkunen-Tiitto, R., Letho, T. and Aphalo, P.J. (2001) *New Phytol.* **150**, 121–131.
- Farman, J.C., Gardiner, B.G. and Shanklin, J.D. (1985) *Nature* **35**, 207–210.
- Flint, S.D., Ryel, R.J. and Caldwell, M.M. (2003) *Agr. Forest Met.* **120**, 177–189.
- Gabeřšcik, A., Vořcina, M., Trost, T., Germ, M. and Björn, L.O. (2002) *J. Photoch. Photobiol. B* **66**, 30–36.
- González, R., Wellburn, A.R. and Paul, N.D. (1998) *Physiol. Plantarum* **104**, 373–378.
- Gröebner, J., Albold, A., Blumthaler, M., Cabot, T., de la Casiniere, A., Lenoble, J., Martin, T., Masserot, D., Müller, M., Philipona, R., Pichler, T., Pougatch, E., *et al.* (2000) *J. Geophys. Res.* **105**, 26991–27003.
- Gwynn-Jones, D., Lee, J.A. and Callaghan, T.V. (1997) *Plant. Ecol.* **128**, 242–249.
- Harborne, J.B. and Grayer, R.J. (1993) In: *The Flavonoids: Advances in Research since 1986* (Ed. by J.B. Harborne), pp. 589–618. Chapman & Hall, London.
- Hughes, K.A., Scherer, K., Svenøe, T., Rettberg, P., Horneck, G. and Convey, P. (2006) *Soil Biol. Biochem.* **38**, 1488–1490.
- Håkansson, K.B. (2006) *Påvirker det nuværende niveau af ultraviolet-B stråling Salix arctica? - Et eksklusions forsøg fra Nordostgrønland*. M.Sc. thesis, Department of Terrestrial Ecology, Biological Institute, University of Copenhagen.
- Johanson, U., Gehrke, C., Björn, L.O. and Callaghan, T.V. (1995a) *Funct. Ecol.* **9**, 713–719.
- Johanson, U., Gehrke, C., Björn, L.O., Callaghan, T.V. and Sonesson, M. (1995b) *Ambio* **24**, 106–111.
- Johnson, D. (2003) *Res. Microbio.* **154**, 315–320.
- Johnson, D., Campbell, C.D., Lee, J.A., Callaghan, T. and Gwynn-Jones, D. (2002) *Nature* **416**, 82–83.
- Jokela, K., Leszczynski, K. and Visuri, R. (1993) *J. Photoch. Photobiol. B* **58**, 559–566.
- Jones, M.H., Macdonald, S.E. and Henry, G.H.R. (1999) *Oikos* **87**, 129–138.
- Lehrner, G., Delatorre, J., Lütz, C. and Cardemil, L. (2001) *J. Photoch. Photobiol. B* **64**, 36–44.
- Madronich, S., McKencie, R.L., Björn, L.O. and Caldwell, M.M. (1998) *J. Photoch. Photobiol. B* **46**, 5–19.
- Paul, N. (2001) *New Phytol.* **150**, 1–8.
- Phoenix, G.K. and Lee, J.A. (2004) *Ecol. Res.* **19**, 65–74.

- Phoenix, G.K., Gwynn-Jones, D., Lee, J.A. and Callaghan, T.V. (2000) *Plant Ecol.* **146**, 67–75.
- Phoenix, G.K., Gwynn-Jones, D., Callaghan, T.V., Sleep, D. and Lee, J.A. (2001) *J. Ecol.* **89**, 256–267.
- Phoenix, G.K., Gwynn-Jones, D., Lee, J.A. and Callaghan, T.V. (2002) *Plant. Ecol.* **165**, 263–273.
- Rinnan, R., Keinänen, M.M., Kasurinen, A., Asikainen, J., Kekki, T.K., Holopainen, T., Ro-Poulsen, H., Mikkelsen, T.N. and Michelsen, A. (2005) *Global Change Biol.* **11**, 564–574.
- Robberecht, R., Caldwell, M.M. and Billings, W.D. (1980) *Ecology* **61**, 612–619.
- Robson, T.M., Pancotto, V.A., Flint, S.D., Ballaré, C.L., Sala, O.E., Scopel, A.L. and Caldwell, M.M. (2003) *New Phytol.* **160**, 379–389.
- Rousseaux, M.C., Ballaré, C.L., Giordano, C.V., Scopel, A.L., Zima, A.M., Szwarcberg-Bracchitta, M., Searles, P.S., Caldwell, M.M. and Diaz, S.B. (1999) *P. Natl. Acad. Sci. USA* **96**, 15310–15315.
- Rousseaux, M.C., Julkunen-Tiitto, R., Searles, P.S., Scopel, A.L., Aphalo, P.J. and Ballaré, C.L. (2004a) *Oecologia* **138**, 505–512.
- Rousseaux, M.C., Flint, S.D., Searles, P.S. and Caldwell, M.M. (2004b) *Photoch. Photobiol. B* **80**, 224–230.
- Rozema, J., Björn, L.O., Bornman, J.F., Gaberscik, A., Häder, D.-P., Trost, T., Germ, M., Klisch, M., Gröniger, A., Sinha, R.P., Lebert, M., He, Y.-Y., *et al.* (2002) *J. Photoch. Photobiol. B* **66**, 2–12.
- Rozema, J., Boelen, P. and Blokker, P. (2005) *Environ. Pollut.* **137**, 428–442.
- Rozema, J., Boelen, P., Solheim, B., Zielke, M., Buskens, A., Doorenbosch, M., Fijn, R., Herder, J., Callaghan, T., Björn, L.O., Gwynn-Jones, D., Broekman, R., *et al.* (2006) *Plant. Ecol.* **182**, 121–135.
- Ruhland, C.T. and Day, T.A. (2000) *Physiol. Plantarum* **109**, 244–351.
- Ruhland, C.T., Xiong, F.S., Clark, W.D. and Day, T.A. (2005) *Photoch. Photobiol. B* **81**, 1086–1093.
- Searles, P.S., Flint, S.D. and Caldwell, M.M. (2001a) *Oecologia* **127**, 1–10.
- Searles, P.S., Kropp, B.R., Flint, S.D. and Caldwell, M.M. (2001b) *New Phytol.* **152**, 213–221.
- Semerdjieva, S.I., Sheffield, E., Phoenix, G.K., Gwynn-Jones, D., Callaghan, T.V. and Johnson, G.N. (2003a) *Plant Cell Environ.* **26**, 957–964.
- Semerdjieva, S.I., Phoenix, G.H., Hares, D., Gwynn-Jones, D., Callaghan, T.V. and Sheffield, E. (2003b) *Physiol. Plantarum* **117**, 289–294.
- Sigsgaard, C., Petersen, D., Grøndahl, L., Thorsøe, K., Meltøfte, H., Tamstorf, M. and Hansen, B.U. (2006) In: *Zackenberg Ecological Research Operations, 11th Annual Report, 2005* (Ed. by M. Rasch and K. Caning), pp. 11–35, Danish Polar Center, Ministry of Science, Technology and Innovation, Copenhagen.
- Solheim, B., Johanson, U., Callaghan, T.V., Lee, J.A., Gwynn-Jones, D. and Björn, L.O. (2002) *Oecologia* **133**, 90–93.
- Strasser, R.J., Tsimilli-Michael, M. and Srivastava, A. (2004) In: *Chlorophyll a Fluorescence: A signature of photosynthesis*, Advances in Photosynthesis and Respiration (Ed. by G.C. Papageorgiou and Govindjee), pp. 321–363. Volume 19.
- Wardle, D.A. (2002) *Monographs in Population Biology* **34**. Princeton University Press, Princeton, New Jersey.
- Webb, A.R. (1997) In: *Plants and UV-B: Responses to Environmental Change* (Ed. by P.J. Lumsden), pp. 13–30. Cambridge University Press, United Kingdom.
- Weatherhead, B., Tanskanen, A., Stevermer, A., Andersen, S.B., Arola, A., Austin, J., Bernhard, G., Browman, H., Fioletov, V., Grewe, V., Herman, J.,

- Josefsson, W., *et al.* (2005) In: *ACIA, Arctic Climate Impact Assessment* (Ed. by C. Symon, L. Arris and B. Heal), pp. 151–182. Cambridge University Press, Cambridge.
- WMO (2003) *Scientific Assessment of Ozone Depletion: 2002. Global Ozone Research and Monitoring Project*. Report no. 47, World Metrological Organization, Geneva.
- Xiong, F.S. and Day, T.A. (2001) *Plant Physiol.* **125**, 738–751.
- Zaller, J.G., Caldwell, M.M., Flint, S.D., Scopel, A.L., Osvaldo, E.S. and Ballaré, C.L. (2002) *Global Change Biol.* **8**, 867–871.
- Zelles, L. (1999) *Biol. Fert. Soils* **29**, 111–129.

Paper VII

**Ambient UV-B doses decreases PSII performance and net
photosynthesis under natural irradiance in high arctic *Salix arctica*
heath.**

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Ambient UV-B doses decreases PSII performance and net photosynthesis under natural irradiance in high arctic *Salix arctica* heath

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Abstract

Ambient UV-B potentially impacts the photosynthetic performance of high arctic plants, particularly in years with earlier snowmelt. We conducted an UV-exclusion experiment in a dwarf scrub heath in NE Greenland (74°N), with open control, filtered control, and UV-B and UV-AB reduction treatments, all in combination with leaf angle control. Photosynthetic performance at the leaf level was characterized with simultaneous gas-exchange and chlorophyll fluorescence measurements. Photosystem II (PSII) behavior through the growing season was investigated with fluorescence measurements. End season harvest allowed for determination of specific leaf area and content of leaf carbon, nitrogen and UV-B absorbing compounds. Ambient UV-B reduced net photosynthesis in *Salix arctica* leaves fixed in a position exposing them to maximum irradiance, with ca 60% UV-B reduction as reference. Ambient UV-B decreased both Calvin Cycle capacity, i.e. maximum rate of electron transport (V_{cmax}) and maximum carboxylation rate of Rubisco (J_{max}), and the PSII performance, i.e. decreased quantum yield and increased energy dissipation processes. The parallel decrease in PSII performance in leaves with natural random leaf angles on two similar sites signifies that this response may be extended to all leaves across random position and angle in the vegetation. These findings add further evidence that ambient UV-B currently is an important stress factor for plants in high arctic Greenland.

Keywords: UV-B, PSII performance, photosynthesis, High Arctic, *Salix arctica*, Zackenberg, tundra ecosystem

Introduction

The stratospheric ozone layer reduces the solar UV-B radiation (280-315 nm) reaching the earth surface (Kerr 1993; McKenzie, Connor & Bodeker 1999). The ozone layer over the Arctic is projected to recover to pre-1960s levels around 2050 (Shindell, Rind & Lonergan 1998; Taalas, Kaurola & Kylling 2000). However, since stratosphere cooling by greenhouse gasses is increasing the formation of polar stratospheric clouds, which are sites of ozone-destroying reactions and elevated UV-B levels are projected to persist over the Arctic for several decades (WMO 2003; Weatherhea, Tanskanen & Stevermer 2005). At the same time an earlier snow melt and start of growing season due to warming is likely to expose the vegetation to the higher UV-B radiation generally occurring in the spring. Also vegetation located in the snow-free patches will particular receive increased UV-B dose due to irradiance reflected from the surrounding snow (Jokela, Leszczynski & Visuri 1993; Gröebner *et al.* 2000). In high arctic NE Greenland where this study takes place, the growing season is in some years substantially extended in response to the warming (Høye *et al.* 2007). The UV-B fluxes in NE Greenland usually peak in June causing high variability in total growing season exposure. Growing conditions for high arctic plants are harsh as exposed to an extreme environment with a short growing season, low temperatures and nutrient limitation. Studies on ecological responses to UV-B radiation in the Arctic are a research area of growing interest (Callaghan *et al.* 2004a; 2005), with widely variable and often limited responses of plants to perturbations. Considerable tolerance towards effects of UV-B has been reported for some subarctic and arctic dwarf shrubs (Phoenix *et al.* 2000; 2002; Callaghan *et al.* 2004b; Rozema *et al.* 2006). Moreover, as discussed by Phoenix *et al.* (2001) and Phoenix & Lee (2004),

effects of other climatic changes such as increased CO₂, temperature or changes in precipitation may overwhelm the detrimental effects of enhanced UV-B in the sub arctic. However, little attention has been paid to the photosynthetic performance in natural ecosystems in relation to ambient solar UV-B radiation in the Arctic (Albert *et al.* 2008b).

We hypothesize that if the present UV radiation affects the vegetation significantly, then reduction of the irradiance load would improve the photosynthetic performance of the plants. Therefore an experiment where ambient UV-irradiance is reduced substantially by means of filters was established in NE Greenland. This approach allows directly results to the impact of the current level of UV-radiation, including the variability of exposure during the growing season and from year to year. Earlier studies in the area, with similar setup, have reported reduced root biomass in *Vaccinium uliginosum*, and changes in microbial community composition in ambient UV-B (Rinnan *et al.*, 2005). We have also found decreased PSII performance in spite of increased UV-B screening compounds in *Salix arctica* and *Vaccinium uliginosum* (Albert, Mikkelsen & Ro-Poulsen 2005; Bredahl, Ro-Poulsen & Mikkelsen 2004) and decreased net photosynthesis in *Vaccinium uliginosum* (Albert *et al.* 2005). Leaf angle control to homogenize the received irradiance led to observations of increased energy dissipation and a reduced electron transport to the Calvin Cycle in ambient UV-B (Albert *et al.* 2008a). In order to relate results directly to other more traditional photosynthetic parameters, e.g. quantum yield, non-photochemical quenching, maximum rate of electron transport, maximum rate of Rubisco carboxylation and photosynthetic light response, we here combine gas-exchange and fluorescence measurements simultaneously on *Salix arctica*. To investigate the importance of

manipulating the leaf exposure, the PSII performance was followed in parallel through most of the growing season on two additional sites with leaves in their natural leaf angle positions in the vegetation cover.

Materials and Methods

Experimental site

The fieldwork was carried out in a high arctic heathland at Zackenberg Research Station, North East Greenland, (74°N; 21°E) in July and August 2005.

The plant species investigated was the dominant dwarf shrub *Salix arctica* Pall. Lge. (Böcher *et al.* 1978), which has a robust stem and broad leaves well suited for the manipulations and measurements.

Experimental set-up and treatments

To compare *Salix* responses to treatment at different microclimatic conditions and across a random distribution of leaf angles two sites were established in 2001. Site 1 (level site) was situated on almost level ground, whereas Site 2 (sloping site) were situated on a 40°-45° south facing slope (Bredahl *et al.* 2004). These sites were established with permanent filter plots, 60x40 cm² and 5 cm above surface plus open controls, and maintained during most of the growing seasons 2001-2003, left unmaintained in 2004 and reestablished in 2005. Both sites are co-dominated by *Vaccinium uliginosum* and *Salix arctica*.

To reduce the variability and maximize the naturally irradiance doses a third site (fixed angle site) was established in the period 20th June to 30th July. Nylon strings were used to fix plant-shoots and leaves to rectangular aluminum frames. Each frame was forced into

the soil just in front of a *Salix* plant base. A long (10-20 cm) shoot was carefully fixed in a way so that all leaf surfaces were exposed in an angle of 45° facing south. Finally the filter sheets were fastened to the frames with clamps. This setup exposed the plants to maximum PAR and UV-B and allowed easy access for measurements (Albert *et al.* 2005).

In all sites the experimental design was parallel, with four randomized blocks which included all four treatments (C, F, UVB and UVAB, see below). However, on site 3 each of the blocks consisted of 20 individual shoots on separate plants, where each treatment was applied on 5 different shoots (80 shoots in total).

The treatments consisted in reducing the UV-irradiance with ambient UV-B irradiance as reference. This reduction was achieved by filtering the solar radiation through filters, Mylar® (type D, DuPont Teijin Films, Wilmington, Delaware, USA; Treatment UV-B) and Lexan® (RIAS, Roskilde, Denmark; Treatment UV-AB). In general, the Mylar filter transmits $\lambda > 320$ nm (Cybulski & Peterjohn 1999) and Lexan transmits $\lambda > 400$ nm (Bredahl *et al.*, 2004). As controls were used one treatment without filter – an open control (treatment C), and another with PAR transparent filter, Teflon® (Fluoretek AB, Knivsta, Sweden; Treatment F). Teflon transmits $\lambda > 280$ nm (Cybulski & Peterjohn 1999). Measurements with a broad-band cosine corrected UV-B sensor (UV-S-310-T, Scintec, Atmosphärenmesstechnik GmbH, Tübingen, Germany – now manufactured as UV-S-B-T from Kipp & Zonen B.V., Delft, The Netherlands) showed that the plant canopy under Teflon, Mylar and Lexan was exposed to approximately 91%, 39% and 17% of the clear sky control UV-B irradiance, and 97%, 90%, 91% of the photosynthetically active radiation (PAR) respectively, slightly depending on the

exposure angle to the sun (Bredahl *et al.* 2004). No significant filter degradation after a full growth season was observed by Bredahl *et al.* (2004) but filters were changed at the start of each growing season. No microclimatic differences in filtered treatments were recorded on the temperatures and humidity in air and soil, but the soil temperature was increased by 0.3-1.0°C in filtered treatments versus the open control (Bredahl *et al.* 2004).

Climatic data

Climatic data were recorded at the nearby climatic station on the Zackenberg Field station throughout the season. UV-B and PAR data were obtained from the meteorological station at Zackenberg (no. 641, ASIAQ, Greenland Survey). Hourly means of the current UV-B flux was recorded by a horizontally mounted erythema-weighted UV-Biometer (501A, Solar Light Co. Inc., Philadelphia, PA, USA) and the incoming PAR irradiance with a LI-190SA (Li-Cor, Nebraska, NE, USA) in 2m height. The unit for UV-B is mean erythemal dosis (MED, Watt/m²) and for PAR $\mu\text{mol}/\text{photons}/\text{m}^2$. Data were provided from ClimateBasis via the Zackenberg Database (<http://zdb.dmu.dk>).

Simultaneous measurements of leaf gas-exchange and fluorescence

Leaf gas-exchange of CO₂ and H₂O together with chlorophyll fluorescence was measured on fully developed leaves with a Licor, LI-6400, equipped with a fluorescence leaf cuvette (6400-40 Leaf Chamber Fluorometer, LI-COR Biotechnology, Lincoln, Nebraska USA) during an intensive campaign in mid-July (19th to 26th July) alternating between filter control, F, and UVB screened treatment, UV-B. No measurements were done in the

open control and UVAB. After initial trials and testing we chose the following protocols for measuring CO₂ and light response curves.

CO₂ response were measured after 5 min acclimation at CO₂ = 400 ppm and saturating light (PAR = 1200 $\mu\text{mol photons} / \text{m}^2 / \text{s}$) where after the CO₂ level was stepped down from 400 to 300, 200, 100 and 50 ppm CO₂ with ca. 2 min intervals and then taken back to 400 ppm for 4 minutes re-acclimation until initial steady state was reached again. Hereafter the CO₂ level was stepped up to 600, 800, 1000, 1200, 1400 and 1600 ppm. Measurements were recorded when the intercellular CO₂ concentration, C_i, and net photosynthesis, P_n, was stable (Coefficient of variation (CV) < 2% over 10 seconds) at each CO₂ level.

Light response curves were initiated after 5 min acclimation to CO₂ = 400 ppm and PAR = 1200 $\mu\text{mol photons} / \text{m}^2 / \text{s}$. Light was then stepped down from 1800 to 1600, 1400, 1200, 1000, 800, 600, 400, 200, 100, 50 and 0 $\mu\text{mol photons} / \text{m}^2 / \text{s}$ with ca. 2 min at each step when C_i and P_n were stable (CV < 2% over 10 seconds). At the end of each step the steady state fluorescence, F_s', and gas-exchange parameters were recorded. Then a saturating light pulse, 6000 $\mu\text{mol photons} / \text{m}^2 / \text{s}$, were applied to the enclosed leaf area and the maximal fluorescence emission, F_M', at that particular light step was recorded. The light was then shut off for 5 seconds and during the last 2 seconds a far red pulse, depleting the electron transport chain between PSII and PSI, enabled the measurement of the minimum fluorescence in light, F_O'. Light was then turned on again being set to the new light level.

During all measurements the relative humidity, RH, in the cuvette was 45-55 %, cuvette block temperature was set to 19°C. Prior to all measurement loggings a matching of the

IRGA's was done. No changes in the stomatal conductance during measurements of individual CO₂ and light response curves were observed. In total 38 leaf samples were measured, 19 leaf samples from each treatment (F and UVB). Calculation of maximum rate of Rubisco carboxylation, $V_{C,Max}$ and maximum rate of electron transport, J_{Max} , followed the approach of Dubois *et al.* (2007) based on the principles originating from Farquhar *et al.* (1980).

Chlorophyll- a Fluorescence

In addition to the measurements with the fluorescence cuvette, chlorophyll fluorescence was measured with two different approaches to assess PSII performance in detail, both in light and in dark, during most of the growing season. On all sites the Pulse Amplitude Method, PAM (Schreiber 2004), were used, whereas the recording of transients of chlorophyll-a fluorescence from dark induction curves (Strasser, Tsimilli-Michael & Srivastava 2004) was used on fixed angle site only. Measurements were done at weekly intervals on *Salix arctica* with 6 campaigns in all sites, with a total of 80 (4 blocks with 5 measurements in each 4 treatments). The measurements were done site by site and always in the same order, alternating between different treatments one block at a time. Initial measurements were done on June 22nd (level and sloping site) and on June 23rd (fixed angle site) on the plants 3-5 days after filter attachment. Afterwards, the filters were permanently attached and removed only when the measurements were conducted. Last measurements were done 6th (level), 8th (sloping) and 1st (fixed angle) of August.

The PAM measurements were done with a portable chlorophyll fluorometer (PAM-2100, Heinz Walz GmbH, 91090 Effeltrich, Germany). The leaves fully developed were inserted in the leaf clip holder in horizontal position (a small level was attached the leaf clip holder) natural light conditions (no supplementary actinic light) was used and the steady state fluorescence, F_S' , (PAM-2100, Heinz Walz GmbH, 91090 Effeltrich, Germany), was measured. After a saturating pulse of $6000 \mu\text{mol photons/ m}^2/\text{s}$ the maximal fluorescence in light, F_M' , was measured. Minimal fluorescence, F_O' , was measured after a 5 second long dark adaptation by covering the sample with a dark cloth under which far red pulse was given during the last 2 seconds. After 30 minutes of dark adaptation in leaf the clip the maximal, F_M , and minimal, F_O , fluorescence was measured. Transients of chlorophyll-a fluorescence (fixed angle site only) were measured using a Handy PEA (Hansatech Instruments, Ltd. King's Lynn Norfolk, UK) with a 12-bit resolution from $10 \mu\text{s}$ to 1s and a time resolution of $10 \mu\text{s}$ for the first 200 data points (Strasser, Srivastava & Govindjee 1995) after leaves were dark adapted for another 30 minutes. The excitation light intensity was close to $3000 \mu\text{mol photons/ m}^2/\text{s}$ from an array of six red light emitting diodes with a peak wavelength at 650 nm focused on the leaf surface in the clips on a spot of 4 mm diameter. The chlorophyll-a fluorescence signals were detected by the Handy Pea using a PIN photocell after having passed through a long pass filter (50% transmission at 720 nm) (Srivastava *et al.* 1997).

Definitions of fluorescence parameters

From the PAM measurements the maximum quantum yield (in dark) were calculated from the maximal fluorescence F_M and minimum fluorescence F_O as $F_V/F_M = (F_M - F_O)/F_M$

(Schreiber 2004). For all light levels the quantum yield were calculated from the maximal fluorescence at each light step F_M' and minimum fluorescence F_O' as $F_V'/F_M' = (F_M' - F_O')/F_M'$, Stern-Volmer non-photochemical quenching as $NPQ = (F_M - F_M')/F_M'$, non-photochemical quenching coefficient as $qN = 1 - (F_M' - F_O')/(F_M/F_O)$ and the photochemical quenching coefficient as $qP = (F_M' - F_S')/(F_M'/F_O')$ (Schreiber, 2004).

From the fluorescence transient, the measured parameters (F_O , F_M , t_{FM} , Area, $F_{50\mu s}$, $F_{100\mu s}$, $F_{300\mu s}$, F_J , F_I) led to calculation and derivation of a range of parameters (JIP test) according to Strasser *et al.* (2004). See Albert *et al.* (2005) for a summary of all parameters, formulae, definition of symbols. Careful translation of the measured parameters into JIP test parameters provides information of the stepwise flow of energy through PSII at different levels: 1) specific fluxes on the level per reaction centre (RC) for absorption (ABS/RC), trapping (TR_0 /RC), dissipation (DI_0 /RC) and electron transport (ET_0 /RC) and 2) phenomenological fluxes on the level of the excited sample cross section (CS) for absorption (ABS/CS), trapping (TR_0 /CS), dissipation (DI_0 /CS) and electron transport (ET_0 /CS). By assuming that F_0 and F_M are reasonable measures of the absorption energy flux per excited cross section of leaf sample (ABS/CS) in arbitrary units of a particular leaf sample in the dark adapted state, phenomenological fluxes were estimated from both F_0 and F_M , respectively. The specific and phenomenological fluxes are interrelated by the quantum efficiencies, which are 1) the maximum quantum yield of primary photochemistry (F_V/F_M) which in this terminology is equal to the efficiency by which an absorbed photon will be trapped by the PSII RC with the resultant reduction of Q_A to Q_A^- (TR_0 /ABS) and 2) the efficiency by which a trapped exciton, having triggered the reduction of Q_A to Q_A^- can move an electron further than Q_A^- into the intersystem

electron transport chain (ET_0/TR_0). Integrative parameters, so called performance indexes reflecting performance of the overall energy flow processing are 1) based on quantum efficiencies and hereby related to the situation of assuming equal absorption (PI_{ABS}) and 2) based on the phenomenological fluxes related to the per cross section level (PI_{CS_0} and PI_{CS_M}). Estimation of the density of active PSII reaction centers per cross section (RS/CS_0 and RC/CS_M) is also possible. Brief parameter descriptions are given in the results section the first time a parameter occurs.

Leaves

After the last measurements all leaves were harvested in order to determine biomass, leaf area, the content of UVB absorbing compounds and concentration of carbon and nitrogen. Digital photographs were taken of all the filter covered leaves beside a brick of known area immediately after the end harvest of the season on August 10th 2005. The photographs were converted to black and white and loaded in a pixel counting program (Bitmap, S. Danbæk, Department of Biology, University of Copenhagen) and the leaf areas were determined. The dry weight was determined after oven drying at 80°C.

UV-B absorbing compounds were extracted as described in Albert *et al.* (2005). Further, total C and N were determined on a Leco Truespec CN elemental determinator (Leco Corporation, Michigan, USA).

Statistical analysis

Analysis of variance was performed using a general linear model (Proc GLM) for both the random sampling and the repeated measures (Statistical Analysis Systems Institute,

2008). Fluorescence variables measured with the PAM and HandyPea, on fixed angle site only, were tested with a repeated measurement ANOVA, with treatment as factor and day repeated on each subject being the individual leaves. This was to take into account that the same experimental unit i.e. the leaves on the same shoot was measured several times during the season. On all sites the effects of treatment were also tested separately for each day of measurement. In addition, along with the effects of treatments the factors date and site were tested across all measurements during the season. Repeated measures general linear model (Proc GLM) was used to test for effects of treatment on leaf gas-exchange and fluorescence parameters from the light- and CO₂ response curves whereas a general linear model for effects of treatment on leaf biomass, specific leaf area (SLA), UVB absorbing compounds, C and N to test. Tukey's test was used to compare treatment differences ($P < 0.05$) relative to each other. Levene's test was used to test for homogeneity of variance. Where necessary, parameters were transformed in order to meet the assumptions of ANOVA. All values presented here are non-transformed. Multivariate ANOVA (MANOVA's) were conducted prior to ANOVA's in order to investigate if true value overall responses to treatment and to validate that significance in ANOVA were not due to chance.

Results

Climatic

UV-B irradiance during the growth season in Zackenberg accumulated to 15.20, 13.24, 9.95 MED/ month and maximum was 0.082, 0.073, 0.069 MED/ hour in June, July and

August 2005. In June, July and August the average PAR irradiance was 918, 817 and 484 $\mu\text{mol photons/ m}^2/\text{s}$ and the maximum was 1454, 1512 and 1356 $\mu\text{mol photons/ m}^2/\text{s}$.

Parallel gas-exchange and fluorescence

CO_2 response curves showed decreased net photosynthesis across the full A/C_i -curve in ambient UV-B (Figure 1, $P<0.0001$), no effect on stomatal conductance was seen (Figure 1, $P<0.7698$). Maximum rate of Rubisco carboxylation (V_{cmax}) and maximum rate of electron transport (J_{max}) was lower in ambient UV-B (F), mean \pm standard error being $V_{\text{cmax}} = 51.8 \pm 3.9$ and $J_{\text{max}} = 118.0 \pm 5.9$ compared to the UV-B screened treatment (UV-B) where $V_{\text{cmax}} = 69.4 \pm 4.4$ and $J_{\text{max}} = 141.3 \pm 7.7$ ($P<0.0001$). The calculated rate of non-photorespiratory respiration (R_d) and Michealis-Menten constants for Rubisco for O_2 (K_o) or CO_2 (K_c) did not differ between treatments.

Light response curves (at ambient CO_2 level) revealed decreased net photosynthesis ($P<0.0002$) in ambient UV-B, with increasing differences at PAR levels higher than 400 $\mu\text{mol photons/ m}^2/\text{s}$ (Figure 2). No change in stomatal conductance (and transpiration, not shown) was observed, but intercellular CO_2 concentration was increased in ambient UV-B (Figure 2, $P<0.0002$). Measured in parallel to the gas-exchange the fluorescence in light showed decreased F_s' , F_o' , and in particular F_M' (all $P<0.0001$) in ambient UV-B (Figure 3). This led to decreased photochemical quenching coefficient, qP , and off the quantum yield in light, F_v'/F_M' , in ambient UV-B (Figure 3, both $P<0.0001$). The treatment differences in F_v'/F_M' increased for PAR levels higher than 400 $\mu\text{mol photons/ m}^2/\text{s}$. In the dark measurements, a decreased F_M ($P<0.0001$) and no change in F_o led to a

decreased maximal quantum yield, F_v/F_m , ($P < 0.0001$) in ambient UV-B (data not shown). Relating the light and dark parameters with the Stern-Volmer non-photochemical quenching parameter, NPQ, a higher non-photochemical quenching was seen in ambient UV-B (Figure 3, $P < 0.0001$). Also the non-photochemical quenching coefficient, q_N , was increased in ambient UVB (Figure 3, $P < 0.0001$).

Dark induction curves from the S3 were analyzed according to the JIP-test. An overview of the measured and selected significantly affected parameters can be seen in Figure 4, where all values are indexed relative to the ambient UV-B (F , set to 1). The fluorescence rise from initial level F_0 to $F_{300\mu s}$ showed no differences between treatments, but hereafter the differences at the F_J , F_I and F_M steps increased (Table 1 and Figure 4, $P < 0.0007$, $P < 0.0001$ and $P < 0.0001$), the open control and filtered control being lower than reduced UV-AB and UV-B treatments. An increased area under the induction curve was seen in UV-AB and UV-B treatments. An increased area under the induction curve was seen in UV-AB and UV-B reduced treatments compared to open- and filter controls (Table 1, $P < 0.0001$), whereas the time to reach maximal fluorescence tF_M did not differ. The maximal quantum yield for primary photochemistry, F_v/F_m , which in this terminology is equal to the trapping probability, $TR_0/ABS = \phi P_0$, expresses the probability that an absorbed photon will be trapped by the PSII RC and lead to reduction of one Q_A . This was reduced in F and C compared to UV-AB and UV-B (Table 1, $P < 0.0001$). The probability that a photon trapped by the PSII RC enters the electron transport chain, $ET_0/TR_0 = \psi E_0$, was not significantly different between all treatments although raw values was slightly higher in UV-AB and UV-B screened treatments. The Performance Index on absorption basis, PI_{ABS} , and performance indexes per leaf cross section, PI_{CSm} and PI_{CS0} , were increased in UV-AB and UV-B screened treatments compared to open

and filter control (All $P < 0.0001$). No significant differences were seen in the specific fluxes (absorption, trapping, dissipation and electron transport per PSII RC). The phenomenological fluxes per leaf cross section were affected. Assuming the absorption to be equal to maximal fluorescence, $F_M = CS_M$, this led to a higher trapping, TR_O/CS_m ($P < 0.0001$), equal dissipation, DI_O/CS_m , higher electron transport, ET/CS_m ($P < 0.0001$), per leaf cross section and increased density of active PSII RC's, RC/CS_m ($P < 0.0001$), in UV-AB and UV-B screened treatments compared to open and filtered controls (Table 1, Figure 5). Using F_O as a proxy for absorption, setting $F_O = CS_O$, this led to a higher trapping, TR_O/CS_O ($P = 0.0007$), decreased dissipation, DI_O/CS_O ($P = 0.0037$), higher electron transport, ET/CS_O ($P = 0.0007$), per leaf cross section and increased density of active PSII RC's, RC/CS_O ($P < 0.0001$), in UV-AB and UV-B screened treatments compared to open and filtered control (Table 1, Figure 5).

Leaf characteristics and fluorescence responses from all sites

During the growth season the magnitudes of the maximum quantum yield (F_V/F_M) reflected the seasonality of photosynthetic performance, with initially low values increasing to a peak in mid July. Thereafter senescence processes probably was responsible for the later lower values (data not shown). In response to treatments maximum quantum yield, F_V/F_M , were decreased in open and filtered control compared to UV-AB and UV-B reduced treatments on each of the three sites (all $P < 0.0001$), but the level of F_V/F_M did not differ between sites (Table 2). The underlying explanation for the decreased F_V/F_M was an unaffected (on fixed angle site) to minor changed level of F_O (sloping site, $P = 0.0003$; level site, $P = 0.0075$) and a markedly decreased F_M level in open

and filtered control compared to UV-AB and UV-B reduced treatments on each of the three sites (all $P < 0.0001$). Decreasing level of F_M was seen between sites in the order sloping site > level site > fixed angle site ($P < 0.0001$).

Steady state fluorescence, F_S' , measured at natural light conditions showed generally little or no response to treatments. Only on fixed angle site the F_S' values in UV-B were little higher than UV-AB and F which again were marginally higher than values in C (Table 2, $P < 0.0032$). On all sites an increased maximal fluorescence in light, F_M' , were seen in the UV-AB and UV-B screened treatments compared to open and filtered controls, but it was only significant on the fixed angle site (Table 2, $P < 0.0001$). Minimal fluorescence in light, F_O' , showed generally little or no response to treatments (Table 2), with no general pattern related to the UV-screened versus controls.

The quantum yield in light, F_V'/F_M' , were decreased in open and filtered controls compared to UV-B and UV-AB screened treatments on sloping and fixed angle sites (Table 2, $P < 0.0001$, $P = 0.0247$), except on S1 where only the UV-AB differed from open and filtered controls (Table 2, $P = 0.0017$). The level of F_V'/F_M' was reduced around 15% on fixed angle compared to level and sloping sites ($P < 0.0001$). F_V'/F_M' was correlated negatively to increasing PAR across all measurements ($r^2 = 0.82$, data not shown).

The level of Stern-Volmer non-photochemical quenching, NPQ, were lowest on the fixed angle site ($P < 0.0001$) where it also showed a stepwise decrease from the level in open to filtered control to UV-AB and to UV-B screened treatments. However, only the UV-B screened treatment differed from open and filtered controls (Table 2, $P < 0.0488$).

The non-photochemical quenching coefficient, q_N , was higher at level and sloping sites compared to fixed angle site, but showed no clear response to treatments at each site.

The photochemical quenching coefficient, qP , were higher at the fixed angle site compared to level and sloping sites. There was no effect of treatment on the fixed angle site, but on level site the qP were marginally increased in UV-AB screened treatment compared to open and filtered control (Table 2, $P=0.0247$). On the sloping the UV-AB screened treatment only differed from open control (Table 2, $P=0.0017$).

Leaf content of UV-B absorbing compounds showed decreased values in the UV-AB and UV-B reduced treatments compared to open and filtered control on each site (Table 2), but only across sites the difference was significant ($P=0.0339$). The content of UV-B absorbing compounds were more than doubled on fixed angle site compared to level and sloping sites ($P<0.0001$). Specific leaf area, SLA, did not respond to treatment. However the values were reduced to more than half on the fixed angle site compared to level and sloping site ($P<0.0001$) implying thicker leaves on the fixed angle site. Leaf carbon content did not respond to treatments and no site differences were seen. Leaf nitrogen was increased in the UV-AB compared to UV-B screened treatment, open and filtered controls on across all sites (Table 2, $P=0.0024$).

Discussion

PSII Performance

All PSII characterizing parameters, e.g. decreased quantum yields (F_V/F_M , F_V'/F_M') and increased energy dissipation (qN , NPQ) point to the negative effect of ambient UV-B. This calls for a detailed analysis of the energy flow related to trapping and dissipation of the energy and contribution to electron transport beyond PSII.

The ratio of F_V'/F_M' are stoichiometrically related to the electron transport rate using the formula $ETR = 0.5 \times \text{leaf absorptivity} \times F_V'/F_M'$ (Genty, Briantais & Baker 1989). Hence, from the light response curves the decreased F_V'/F_M' seems to decrease the amount of energy equivalents available in the chloroplast for Calvin Cycle consumption. Indeed the decrease in qP indicates a larger degree of PSII closure in ambient UV-B. The increasing PSII closure at higher PAR levels adds to the need for dissipation of the incoming energy harvested. Various processes involved in dissipation of excessive energy, understood as energy not used in primary photochemistry, are often referred 'non-photochemical quenching' processes. Although differing in formulation, the $NPQ = q_{N_{SV}}$ and q_N are measures of non-photochemical quenching (Krause & Jahns 2004) and they both were increased in ambient UV-B. This clearly demonstrates a higher proportion of dissipated energy in ambient UV-B. Moreover the magnitude of non-photochemical quenching was increasing with PAR level. The total non-photochemical quenching are the sum of the major components: 1) Energy dependent quenching, q_E , related to ΔpH 2) Quenching related to energy redistribution via state transitions, q_T , and 3) Photo-inhibitory quenching, q_I , which is related to various aspects of photoinhibition (Krause & Jahns, 2004). Typically q_E and q_T have short life times $t_{1/2} = 1$ minute and $t_{1/2} = 5-10$ minutes and q_I more than 30 minutes (Krause & Jahns, 2004). Since we do not have data following the fluorescence recovery from a given light state to dark with such short intervals we can not evaluate q_E and q_T (e.g. as done by Schansker, Toth & Strasser 2006). However, the decreased F_V/F_M after 30-35 min in dark clearly shows that q_I is a major component being persistent in ambient UV-B. For a detailed discussion of the complex mechanisms leading to changes in q_I see Krause & Jahns (2004). However, similar to the photo-

inhibitory light the UV-B radiation adds to these processes leading to degradation of D1 (Aro, Virgen & Andersson 1993; Friso *et al.* 1994a; Telfer & Barber 1994) and D2 (Friso *et al.* 1994b; Mattoo *et al.* 1999), see the discussion in Krause & Jahns (2004) and Tevini (2004).

The JIP- test parameters adds further information and support the above interpretation. In general the behavior of the quantum efficiencies, specific and phenomenological fluxes and performance indexes in this study were almost all negatively affected by ambient UV-B, much similar to the behavior in previous experiments (Albert *et al.* 2005; 2008a).

The integration of the response patterns of individual fluxes per active PSII RC's into the Performance Index, PI_{Abs} (assumes samples are having an equal absorption) shows the less favorable conditions for primary photochemistry in ambient UV-B. Hence, the values on the level of specific fluxes per active PSII RC's were not significantly different, but the absorption flux of photons per active RC, ABS/RC , was higher in ambient UV-B. This may reflect a larger average antenna size per PSII RC and may be related to the conversion of PSII RC into heat sinks or the occurrence of regrouping of antennas from active RC's to inactive RC's (Strasser *et al.* 1995; van Heerden *et al.*, 2003). This leads to a larger maximum trapping rate of excitons per active PSII RC, TR_o/RC , but due to the higher effective dissipation of un-trapped excitons per active PSII RC, DI_o/RC , this summed up to the same level of electron transport per PSII reaction centre, ET_o/RC . Further, by relating this equal electron transport per active PSII RC (ET_o/RC) to the decreased number of active PSII RC's per leaf cross-section, RC/CS_M , a reduced potential of electron transport capacity per leaf cross-section were to be expected in ambient UV-B. This was confirmed when the ABS/CS were approximated, here to F_M

(but also when F_O was chosen as approximation), and the phenomenological fluxes per cross-section for trapping (TR_O/CS_m) were decreased, dissipation (DI_O/CS_m) were unchanged, but potential electron transport (ET_O/CS_m) were decreased about 20% in ambient UV-B. This behavior of phenomenological fluxes per leaf cross-section decreased the performance index PI_{CS_m} and clearly indicates a reduced overall processing of light energy per leaf sample cross-section in ambient UV-B.

Finally concerning the behavior of the quantum efficiencies to the stepwise processing of energy in the PSII apparatus and onwards: The decreased trapping probability of primary photochemistry, $TR_O/ABS (= F_V/F_M)$, in combination with a unaffected probability of the further reduction of electron acceptors in the intersystem electron transport chain between PSII and PSI (ET_O/ABS) led to higher dissipation of the absorbed energy (DI_O/ABS) in ambient UV-B. Further, the measure of end acceptor reduction at the PSI electron acceptor side (RE_O/ABS) was decreased with 10-15% in ambient UV-B compared to UV-B and UV-AB filtered treatments. Independent of approach PSII performance was negatively affected and the response patterns in energy dissipation processes and electron transport are very similar.

Photosynthetic performance

The decreased net photosynthesis at a natural CO_2 level (400 ppm) could potentially be explained by effects on PSII performance (source side) or on effects on performance of e.g. Calvin Cycle activity, CO_2 diffusion changes, etc (sink side). Since the quantum yield in light were decreased in ambient UV-B it is probable that the linear flow of electrons beyond PSII is also decreased leading to a reduced proportion of available

electrons consumable for Calvin Cycle. This alone could account for the effect on the net photosynthesis at almost all irradiance levels if a large proportion of these electrons are being consumed in the Calvin Cycle and not in other potential sinks as water-water cycle (Asada 2000). The co-limitation of vital steps in the Calvin Cycle, the decreased maximum electron transport rate, J_{Max} , leading to decreased Rubisco regeneration and the decrease in maximum rate of Rubisco carboxylation, V_{cmax} , lowers the photosynthetic performance even more in ambient UV-B. The multiple targets in the photosynthetic apparatus may be related both directly and via down-regulation to obtain balance between changes in PSII performance to sink capacity of energy equivalents consumption in the Calvin Cycle and vice versa.

Decreased net photosynthesis have been shown in UV-B exclusion experiments (Albert *et al.* 2008a; Xiong & Day 2001) and for plants exposed to realistic supplemental UV-B irradiance in the field (Baker, Nogués & Allen 1997; Keiller & Holmes 2001; Keiller, Mackerness & Holmes 2003; Teramura & Sullivan 1994), but Allen *et al.* (1999) did not find such effects. Across these diverse studies several mechanisms have been suggested to be possible limitations on photosynthesis. Some studies suggested that PSII is the primary target of UV-B damage, reducing PSII activity (Strid, Chow & Andersson 1990; Melis, Nemson & Harrison 1992) and abundance of D1 (Aro *et al.* 1993; Jansen *et al.* 1996) while other studies have shown that photosynthetic inhibition can occur without any measurable effect on PSII (Middleton & Teramura 1993; Allen *et al.* 1999). UV-B supplementation studies have identified changes in stomatal conductance (Teramura, Tevine & Iwanzik 1983; Negash & Björn 1986), Rubisco content, reductions in capacity for photosynthetic electron transport (J_{max}) and maximum carboxylation rate (V_{cmax})

(Jordan *et al.* 1992; He *et al.* 1993; Baker *et al.* 1997; Allen *et al.* 1997) as possible limitations on photosynthesis. In contrast to our study Nogués & Baker (1995) also using a parallel gas-exchange and fluorescence technique, concluded that UV-B could decrease light saturated net photosynthesis, V_{cmax} and J_{max} in the absence of any decreases of the photosynthetic activity of PS II (F_V/F_M) (Nogués & Baker 1995; Baker *et al.* 1997). They interpret this indicating a loss or inactivation of Rubisco, which is in line with others reporting UV-B induced impact on both Rubisco activity and content (Vu, Allen & Garrard 1984; Strid *et al.* 1990; Jordan *et al.* 1992; Greenberg *et al.* 1996).

However, criticism have been expressed towards experiments caused by unrealistic high UV-B irradiances (Allen *et al.* 1998; Caldwell & Flint 1997) and also difficulties in comparison of results due to different methodologies has been pointed to (i.e. Day & Neale 2002; Flint, Ryel & Caldwell 2003; Rousseaux *et al.* 2004; Albert *et al.* 2008b). Hence, UV-B impacts are not easy to put in ‘correct’ perspective and the effects on the photosynthetic apparatus in balancing the effects of UV-B are therefore likely to differ between studies with different experimental approaches. However as high arctic plants on the northern limit of their distribution and exposed to harsh climatic conditions which may cause the pronounced impact on the photosynthetic performance in our study.

Screening off the negative impact of UV-radiation by UV-B absorbing compounds in the epidermis is one of the most reported responses to UV-B (Searles, Flint & Caldwell 2001). In this study this response was only significant across all sites, but there was much higher content of UV-B absorbing compounds on the fixed angle site compared to level and sloping sites. In combination with the increased leaf thickness (much lower SLA) on the fixed angle site compared to level and sloping sites, this suggest that leaves on fixed

angle site to a larger degree were responding to avoid the impact of UV-radiation. Despite the plasticity, this UV-avoidance response was not sufficient to avoid negative effects on PSII performance on all sites, and on photosynthesis on fixed angle site. The values of F_V/F_M and F_V'/F_M' were higher in the UV-screened treatments (UV-B and UV-AB) versus the open and filtered controls (C, F) on the level and sloping sites compared to fixed angle site. This may be a 'medium' effect of treatment as the negative impact on F_V'/F_M' increases at higher PAR levels. Hence, even though the measurements were performed at the full range of natural PAR levels, most measurements (around 75%) were conducted at PAR levels less than $500 \mu\text{mol photons} / \text{m}^2 / \text{s}$ leading to fewer measurements of low F_V'/F_M' values.

The similarity of treatment responses on the different sites clearly indicate that PSII performance is also affected in natural vegetation where leaves of random leaf angle and in a random position in the vegetation are exposed to a range of different irradiance doses. As shown for fixed angle site where this was the case, the decrease in F_V'/F_M' led to a decreased net photosynthesis in ambient UV-B, why we suggest that a decreased net photosynthesis is probable in high arctic *Salix arctica* in general. However, the impact on ambient UV-B is likely to be gradual, with some leaves being much affected and others less. On one hand it has been pointed out that the vulnerability to UV-B irradiance may be exacerbated in simple canopy systems as in arctic vegetation (Phoenix *et al.* 2000). On the other hand the variability in leaf angle and leaf position in the canopy or position in a varying vegetation structure could potentially reduce the impact of UV-B irradiances via reduction in exposure. For example, although significant responses were seen in PSII performance and UV-B absorbing compounds in *Vaccinium* (Albert *et al.* 2008a), and in

the present study for *Salix*, Bredahl *et al.* (2004) found decreased intercellular CO₂ concentration and stomatal conductance, but no effects on photosynthesis across *Salix* leaves with random leaf angle. Our results demonstrate that results with less variation are found when homogenizing the dose by leaf angle control, as done in this study.

In conclusion, ambient UV-B reduced net photosynthesis in high arctic *Salix arctica* leaves when ambient irradiance doses were maximized. Simultaneous gas-exchange and fluorescence measurements revealed that ambient UV-B decreased both Calvin Cycle capacity via maximum rate of electron transport and maximum carboxylation rate of Rubisco and the PSII performance via decreased quantum yield and increased energy dissipation processes. The parallel decreased PSII performance for *Salix* on three sites signifies this response to be extended to a large proportion of leaves across random position and angle in the vegetation. These findings add further evidence that ambient UV-B is an important stress factor for plants in high arctic Greenland.

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References

Albert K.R., Mikkelsen T.N. & Ro-Poulsen H. (2005) Effects of ambient versus reduced UV-B radiation on high arctic *Salix arctica* assessed by measurements and calculations of chlorophyll-a fluorescence parameters from fluorescence transients. *Physiologia Plantarum* **124**, 208-226.

Albert K.R., Mikkelsen T.N. & Ro-Poulsen H. (2008a) Ambient UV-B radiation decreases photosynthesis in high arctic *Vaccinium uliginosum*. *Physiologia Plantarum* **133**, 199-210.

Albert K.R., Rinnan R., Ro-Poulsen H., Mikkelsen T.N., Håkansson K.B., Arndal M.F. & Michelsen A. (2008b) Solar Ultraviolet-B Radiation (UV-B) at Zackenberg: The Impact on Higher Plants and Soil Microbial Communities. In: Meltofte H, Christensen T, Elberling B, Forchhammer M, Rasch M, eds. Dynamics of a High Arctic Ecosystem, Advances in Ecological Research Volume 40, New York, USA: Elsevier.

Allen D.J., McKee I.F., Farage P.K. & Baker N.R. (1997) Analysis of limitations to CO₂ assimilation on exposure of leaves of two *Brassica napus* cultivars to UV-B. *Plant, Cell and Environment* **20**, 633–640.

Allen D.J., Nouges S., Morison J.I.L., Greenslade P.D., Mcleod A.R. & Baker N.R. (1999) A thirty percent increase in UV-B has no impact on photosynthesis in well-watered and droughted pea plants in the field. *Global Change Biology* **5**, 235-244.

Asada K. (2000) The water-water cycle as alternative photon and electron sinks. *Philosophical Transactions of the Royal Society of London B Biological Sciences* **29**, 1419–1431.

Aro E.M., Virgin I. & Andersson B. (1993) Photoinhibition of Photosystem II in activation, protein damage and turnover. *Biochimica et Biophysica Acta* **1143**, 113–134.

Baker N., Nogués S., Allen D.J. (1997) Photosynthesis and photoinhibition. In: Lumsden PJ, ed. *Plant and UVB: Responses to Environmental Change*. Society for Experimental Biology Seminar Series 64, Cambridge, UK: Cambridge University Press, 233–246.

Bredahl L., Ro-Poulsen H. & Mikkelsen T.N. (2004) Reduction of the ambient UV-B radiation in the high arctic increases F_v/F_m in *Salix arctica* and *Vaccinium uliginosum* and reduces stomatal conductance and internal CO₂ concentration in *Salix arctica*. *Arctic, Antarctic, and Alpine research* **36**, 364-369.

Caldwell M.M. (1968) Solar ultraviolet radiation as an ecological factor in plant life. *Ecological Monographs* **39**, 243-268.

Caldwell M.M. & Flint S.D. (1997) Uses of biological spectral weighting functions and the need of scaling for the ozone reduction problem. *Plant Ecology* **128**, 66-76.

Callaghan T.V., Björn L.O., Chernov Y. et al. (2004a) Uncertainties and recommendations. *Ambio* **33**, 474-479.

Callaghan T.V., Björn L.O., Chernov Y. et al. (2004b) Rationale, concepts and approach to the assesment. *Ambio* **33**, 393-377.

Callaghan T.V., Björn L.O., Chapin T. et al. (2005) Arctic tundra and polar dessert ecosystems. In: Symon C, Arris L, Heal B, Eds. ACIA, Arctic Climate Impact Assessment. New York, USA: Cambridge University Press, 243–352.

Cybulski W.J. & Peterjohn W.R. (1999) Effects of ambient UV-B radiation on the above ground biomass of seven temperate-zone plant species. *Plant Ecology* **145**,175-181.

Day T.A., Neale P.J. (2002) Effects of UV-B radiation on terrestrial and aquatic primary producers. *Annual Review of Ecology and Systematics* **33**, 371-396.

Dubois J.-J.B., Fiscus E.L., Booker F.L., Flowers M.D. & Reid C.D. (2007) Optimizing the statistical estimation of the parameters of the Farquhar-von Caemmerer-Berry model of photosynthesis. *New Phytologist* **176**, 402-414.

Farquhar G.D., von Caemmerer S. & Berry J.A. (1980) A biochemical model of photosynthetic CO₂ carbon dioxide assimilation in leaves of C₃ carbon pathway species.

Planta **149**, 78-90.

Flint S.D., Ryel R.J. & Caldwell M.M. (2003) Ecosystem UV-B experiments in terrestrial communities: a review of recent findings and methodologies. *Agriculture Forest Metrology* **120**, 177-189.

Friso G., Spetea C., Giacometti G.M., Vass I. & Barbato R. (1994^o) Degradation of Photosystem II reaction centre D1 protein induced by UVB radiation in isolated thylakoids. Identification and characterization of C- and N-terminal breakdown products.

Biochimica et Biophysica Acta **1194**, 78–84.

Friso G., Barbato R., Giacometti G.M. & Barber J. (1994b) Degradation of D2 protein due to UV-B irradiation of the reaction centre of Photosystem II. *FEBS Letters* **339**, 217–221.

Genty B., Briantais J.M. & Baker N. (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence.

Biochimica et Biophysica Acta **990**, 87–92.

Greenberg B.M., Wilson M.I., Gerhardt K.E. & Wilson K.E. (1996) Morphological and physiological responses of *Brassica napus* to ultraviolet-B radiation: Photomodification

of ribulose-1,5- biphosphate carboxylase/oxygenase and potential acclimation processes. *Journal of Plant Physiology* **148**, 78–85.

Gröbner J.A., Albold M., Blumthaler T. et al. (2000) Variability of spectral solar ultraviolet irradiance in an alpine environment. *Journal of Geophysical Research* **105**, 26991-27003.

He J., Huang L.-K., Chow W.S., Whitecross M.I. & Anderson J.M. (1993) Effects of supplementary ultraviolet-B radiation on rice and pea plants. *Australian Journal of Plant Physiology* **20**, 129–142.

Høye T.T., Post E., Mølltofte H., Schmidt N.M. & Forchhammer M.C. (2007) Rapid advancement of spring in the High Arctic. *Current Biology* **17**, 449-451.

Jansen M.A.K, Gaba V., Greenberg B.M., Mattoo A.K. & Edelman M. (1996) Low threshold levels of ultraviolet-B in a background of photosynthetically active radiation trigger rapid degradation of the D2 protein of photosystem II. *Plant Journal* **9**, 693-696.

Jokela K., Leszczynski K. & Visuri R. (1993) Effects of Arctic ozone depletion and snow on UV exposure in Finland. *Photochemistry and Photobiology* **58**, 559-566.

Jordan B.R., He J., Chow W.S. & Anderson J.M. (1992) Changes in mRNA levels and polypeptide subunits of ribulose 1,5 biphosphate carboxylase in response to supplementary ultraviolet-B radiation. *Plant, Cell and Environment* **15**, 91–98.

Kerr R.A. (1993) The ozone reaches a new low. *Science* **262**, 1032-1034.

Krause G.H. & Jahns P. (2004) Non-photochemical energy dissipation determined by chlorophyll-a fluorescence quenching: Characterization and function. In: Papageorgiou GC, Govindjee, eds. *Chlorophyll a Fluorescence: A Signature of Photosynthesis*. Advances in photosynthesis and respiration Volume 19. Germany, Berlin: Springer, 463-495.

Keiller D.R. & Holmes M.G. (2001) Effects of long term exposure to elevated UV-B radiation on the photosynthetic performance of five broad leaved tree species. *Photosynthesis Research* **67**, 229-240.

Keiller D.R., Mackerness S.A.-H. & Holmes M.G. (2003) The action of a range of supplementary ultraviolet (UV) wavelengths on photosynthesis in *Brassica napus* L. in the natural environment: effects on PSII, CO₂ assimilation and level of chloroplast proteins. *Photosynthesis Research* **75**, 139-150.

MacKenzie R., Connor B. & Bodeker G. (1999) Increased summertime UV radiation in New Zealand in response to ozone loss. *Science* **285**, 1709-1711.

Mattoo A.K., Giardi M.T., Raskind A. & Edelman M. (1999) Dynamic metabolism of Photosystem II reaction center proteins and pigments. *Physiologia Plantarum* **107**, 454–461.

Melis A., Nemson J.A. & Harrison M.A. (1992) Damage to functional components and partial degradation of Photosystem II reaction centre proteins upon chloroplast exposure to ultraviolet-B radiation. *Biochimica et Biophysica Acta* **1109**, 312-320.

Middleton E.M. & Teramura A.H. (1993) The protective role of flavonol glycosides and carotenoids in protecting soyabean from UV-B damage. *Plant Physiology* **103**, 741-752.

Negash L. & Björn L.O. (1986) Stomatal closure by ultraviolet radiation. *Physiologia Plantarum* **66**, 360-364.

Nogués S. & Baker N.R. (1995) Evaluation of the role of damage to Photosystem II in the inhibition of CO₂ assimilation in pea leaves on exposure to UV-B radiation. *Plant, Cell and Environment* **18**, 781–787.

Phoenix G.K., Gwynn-Jones D., Lee J.A. & Callaghan T.V. (2000) The impacts of UV-B radiation on the regeneration of a sub-arctic heath community. *Plant Ecology* **146**, 67-75.

Phoenix G.K., Gwynn-Jones D., Callaghan T.V., Sleep D. & Lee J.A. (2001) Effects of global change on a sub-arctic heath: effects of enhanced levels of UV-B radiation and increased summer precipitation. *Journal of Ecology* **89**, 256–267.

Phoenix G.K., Gwynn-Jones D., Lee J.A. & Callaghan T.V. (2002) Ecological importance of solar ultraviolet radiation to a sub-arctic heath community. *Plant Ecology* **165**, 263-273.

Phoenix G.K. & Lee J.A. (2004) Predicting impacts of Arctic climate change: Past lessons and future challenges. *Ecological Research* **19**, 65-74.

Rinnan R., Keinänen M.M., Kasurinen A., Asikainen J., Kekki T.K., Holopainen T., Røpoulsen H., Mikkelsen T.N. & Michelsen A. (2005) Ambient UV radiation in the arctic reduces root biomass and alter microbial community composition but has no effect on microbial biomass. *Global Change Biology* **11**, 564-574.

Rousseaux M.C., Flint S.D., Searles P.S. & Caldwell M.M. (2004) Plant responses to current solar UV-B radiation and to supplemented solar ultraviolet-B radiation simulating ozone depletion: An experimental comparison. *Journal of Photochemistry and Photobiology B Biology* **80**, 224-230.

Rozema J., Boelen P., Solheim B. et al. (2006) Stratospheric ozone depletion: High arctic tundra plant growth on Svalbard is not affected by enhanced UV-B after 7 years of UV-B supplementation in the field. *Plant Ecology* **182**, 121–135.

SAS Institute Inc. (2006) SAS/STAT users guide, Version 9.1. Cary, NC, USA.

Schansker G., Toth S.Z. & Strasser R.J. (2006) Dark recovery of the Chl a fluorescence transient (OJIP) after light adaptation: The qT-component of non-photochemical quenching is related to an activated photosystem I acceptor side. *Biochimica et Biophysica Acta – Bioenergetics* **1757**, 787-797.

Searles P.S., Flint S.D. & Caldwell M.M. (2001) A meta-analysis of plant field studies simulating stratospheric ozone depletion. *Oecologia* **127**, 1–10.

Schreiber U. (2004) Pulse-Amplitude-Modulation (PAM) fluorimetry and saturation pulse method: an overview. In: Papageorgiou GC, Govindjee, eds. *Advances in Photosynthesis and Respiration*. Berlin, Germany: Springer, 279-319.

Shindell D.T., Rind D. & Lonergan P. (1998) Increased polar stratospheric ozone losses and delayed eventual recovery owing to increasing greenhouse-gas concentration. *Nature* **392**, 589-592.

Strasser R.T., Srivastava A. & Govindjee. (1995) Polyphasic chlorophyll-a fluorescence transients in plants and cyanobacteria. *Journal of Photochemistry and Photobiology B* **61**, 32-42.

Strasser R.J., Tsimilli-Michael M. & Srivastava A. (2004) Analysis of the chlorophyll-a fluorescence transient. In: Papageogiou GC, Govindjee, eds. *Advances in Photosynthesis and Respiration*. Berlin, Germany: Springer, 321-362.

Strid A., Chow W.S. & Anderson J.M. (1990) Effects of supplementary ultraviolet-B radiation on photosynthesis in *Pisum sativum*. *Biochimica et Biophysica Acta* **1020**, 260–268.

Srivastava A., Guissé B., Greppin H. & Strasser R.T. (1997) Regulation of antenna structure and electron transport in photosystem II of *Pisum sativum* under elevated temperature probed by the fast polyphasic fluorescence transients: OKJIP. *Biochimica et Biophysica acta* **1320**, 95-106.

Taalas P., Kaurola J. & Kylling A. (2000) The impact of greenhouse gases and halogenated species on the future solar UV radiation doses. *Geophysical Research Letters* **27**, 1127-1130.

Telfer A. & Barber J. (1994) Elucidating the molecular mechanisms of photoinhibition by studying isolated Photosystem II reaction centres. In: Baker NR, Bowyer JR, eds. *Author*

Environmental Plant Biology Series; Photoinhibition of photosynthesis: From molecular mechanisms to the field, Oxford, United Kingdom, BIOS Scientific Publishers, 25-49.

Teramura A.H., Tevini M., Iwanzik W. (1983) Effects of ultraviolet-B irradiance on plants during a mild winter stress. I. Effects of diurnal stomatal resistance. *Physiologia Plantarum* **57**, 175-180.

Teramura A.H. & Sullivan J.H. (1994) Effects of UV-B radiation on photosynthesis and growth of terrestrial plants. *Photosynthesis Research* **39**, 463-473.

Tevini M. (2004) Plant Responses to Ultraviolet Radiation Stress. In: Papageorgiou GC, Govindjee eds. Chlorophyll a Fluorescence: A Signature of Photosynthesis. Advances in photosynthesis and respiration Volume 19. Dordrecht, Netherlands, 605-621.

van Heerden P.D.R., Tsimilli-Michael M., Krüger G.H.J. & Strasser R.J. (2003) Dark chilling effects on soybean genotypes during vegetative development: Parallel studies of CO₂ assimilation, chlorophyll-a fluorescence kinetics O-J-I-P and nitrogen fixation. *Physiologia Plantarum* **117**, 476-491.

Vu C.V., Allen D.J. & Garrard L.A. (1984) Effects of enhanced UV-B radiation (280 – 320 nanometers) on ribulose 1,5-bisphosphate carboxylation in pea [*Pisum sativum* cultivar Little Marvel] and soybean [*Glycine max* cultivar Bragg]. *Environmental and Experimental Botany* **24**, 131–144.

Weathershead B., Tanskanen A. & Stevermer A. (2005) Ozone and Ultraviolet radiation. In: Symon C, Arris L, Heal B, eds. ACIA, Arctic Climate Impact Assessment, New York, USA: Cambridge University Press, 151-183.

WMO (2003) Scientific Assessment of Ozone Depletion: 2002. Global Ozone Research and Monitoring Project. Report no. 47. World Meteorological Organization, Geneva, Switzerland, 498 pp

Xiong F.S. & Day T.A. (2001) Effect of Solar Ultraviolet-B Radiation during Springtime Ozone Depletion on Photosynthesis and Biomass Production of Antarctic Vascular Plants. *Plant Physiology* **125**, 738-751.

Figure 1

CO₂ response curves at PAR intensity 1200 $\mu\text{mol photons/m}^2/\text{s}$. (a) Net photosynthesis (P_n) and (b) Stomatal conductance (g_s) is depicted with the mean \pm standard error for each treatment (n=16). Open symbols are ambient UV-B (Filter control, F) and closed are reduced UV-B treatment (Mylar, UV-B). Stomatal conductance did not differ between treatments ($P=0.6858$). Net photosynthesis across all measurements was decreased in ambient UV-B ($P<0.0001$).

Figure 2:

Light response curves at 400 ppm CO₂. (a) Net photosynthesis (P_n), (b) Intercellular CO₂ concentration (C_i) and (c) Stomatal conductance (g_s) are depicted as mean \pm standard error (n=16), where open symbols are ambient UV-B (Filter control, F) and closed are reduced UV-B treatment (Mylar, UV-B). Net photosynthesis was significantly decreased in ambient UV-B (P=0.0002), the intercellular CO₂ was increased in ambient UV-B (P=0.0002) and no effect was seen on stomatal conductance (P=0.8541).

Figure 3:

Chlorophyll fluorescence response of illuminated leaves. Means \pm standard errors in light response curves (n=16), where open symbols are ambient UV-B (Filter control, F) and closed are reduced UV-B treatment (Mylar, UV-B): (a) Upper curves Maximal (F_M'), middle curves minimal (F_O') and lower curves steady state (F_S') fluorescence. (All $P < 0.0001$); (b) Maximum quantum yield, F_V'/F_M' ($P < 0.0001$); (c) Stern-Volmer Non-Photochemical Quenching, NPQ ($P < 0.0001$); (d) Triangles, photochemical coefficient, qP ($P < 0.0001$), Circles, non-photochemical coefficient, qN ($P = 0.026$).

Figure 4: Radar plot of JIP-test parameters from fixed angle site

Means of treatments cross-season are indexed relative to ambient UV-B (F). Symbols for each treatment are crosses for open control (C), open circles are filtered control (F), closed circles are UV-B reduced treatment (UV-B) and closed squares are UV-AB reduced treatment (UV-AB). See materials and methods for parameter presentation and Table 1 for statistics. All data are extracted and derived from the fast fluorescence transient O-J-I-P, according to Strasser et al. (2004). Measured parameters: Minimal fluorescence F_0 ; fluorescence measured at time $100\mu\text{s}$, $F_{100\mu\text{s}}$; $300\mu\text{s}$, $F_{300\mu\text{s}}$; 2 ms, F_J ; 30ms, F_I ; and Maximal fluorescence F_M ; Area under induction curve, Area.

Derived parameters: Quantum yields for trapping TR_O/ABS , dissipation DI_O/ABS , electron transport ET_O/ABS and reduction of end acceptors RE_O/ABS ; ABS , absorption energy flux; CS , excited cross-section of leaf sample; TR , excitation energy flux trapped by the RC and utilized for the reduction of Q_A to Q_A^- ; DI , dissipation energy flux at the level of the antenna chlorophylls; ET , flux of electrons from Q_A^- into the intersystem electron transport chain; RC , PSII reaction centre; RC/CS , concentration of PSII RC per excited CS of leaf sample; PI , performance index.

Table 1: JIP-test parameters

Measured and derived parameters from the fluorescence transients measured in dark. For each treatment (C=open control, F=filtered control, UVB=UV-B screened treatment and UVAB=UV-AB screened treatment) the mean \pm standard error across season are given. Variance analysis was used to test treatment differences with a general linear model including the factors block and date. The p-values are given and differences between means from the Tukey test are given as letters. See parameter description in figure 4 and materials and methods.

Table 2: Flourescence and leaf characteristics from level, sloping and fixed angle sites.

Shown are the mean and standard error across season for each site. P values was obtained by testing effect of treatments (C=open control, F=filtered control, UVB=UV-B screened treatment and UVAB=UV-AB screened treatment) with a general linear model including the factor block followed by Tukey's test for comparisons of treatment means. The model for flourescence parameters included the sampling date and PAR as co-variates. The effect of site was tested across all measurements.

Fig 1

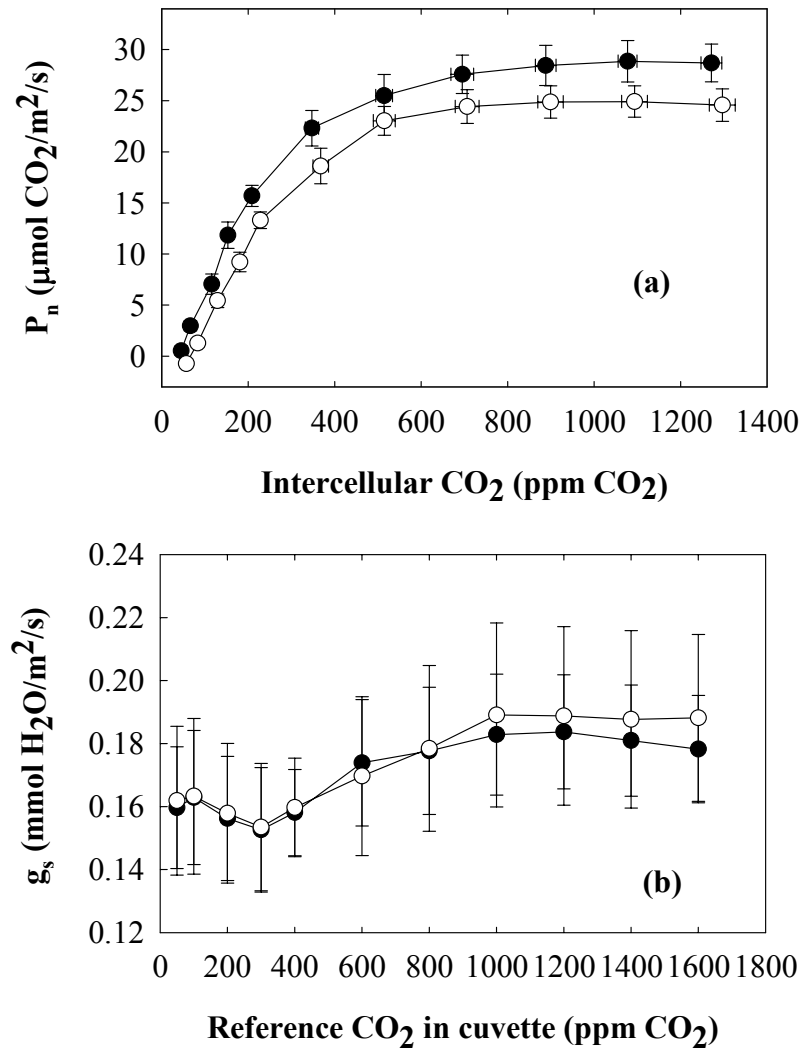


Fig 2

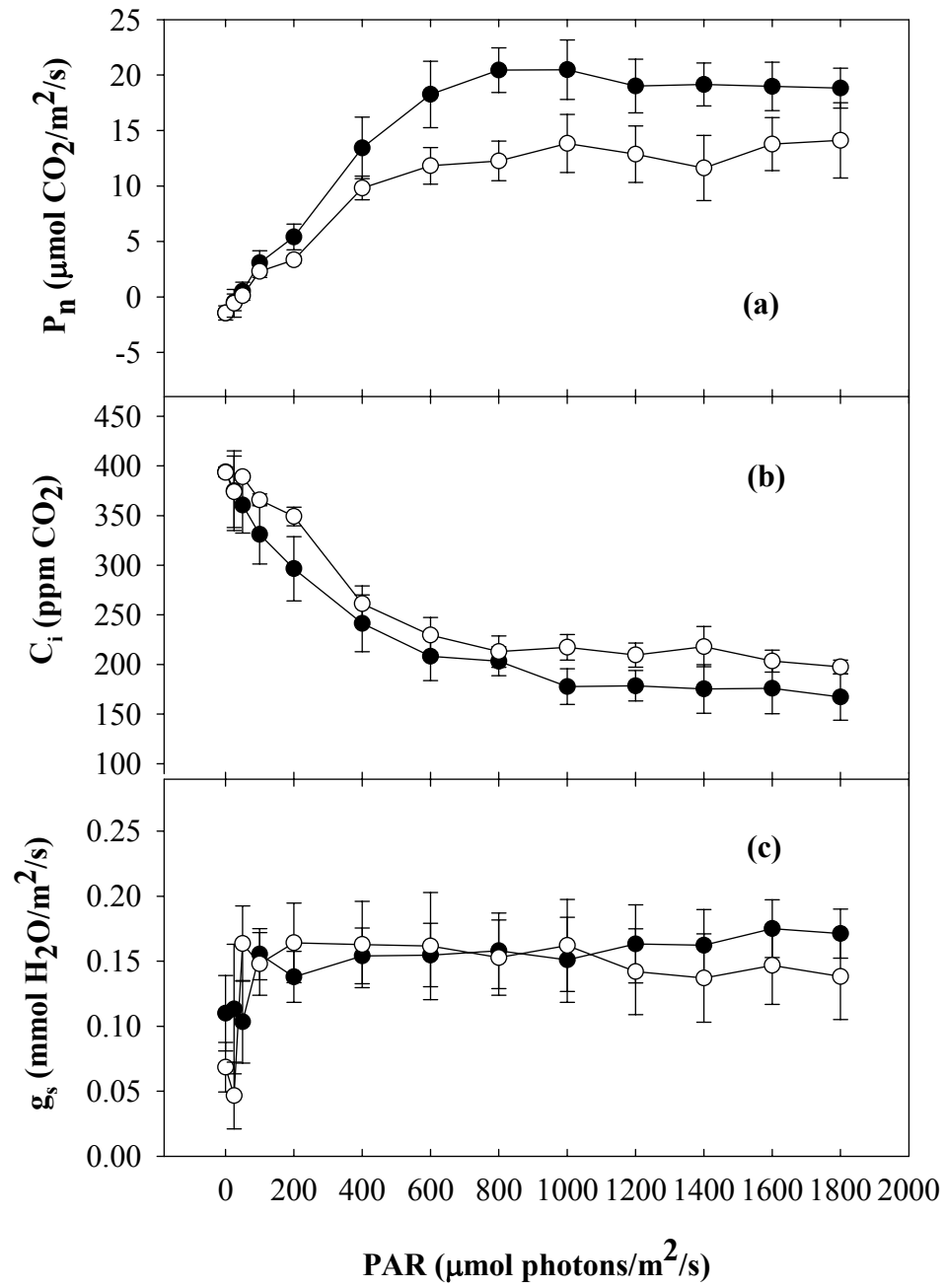


Fig 3

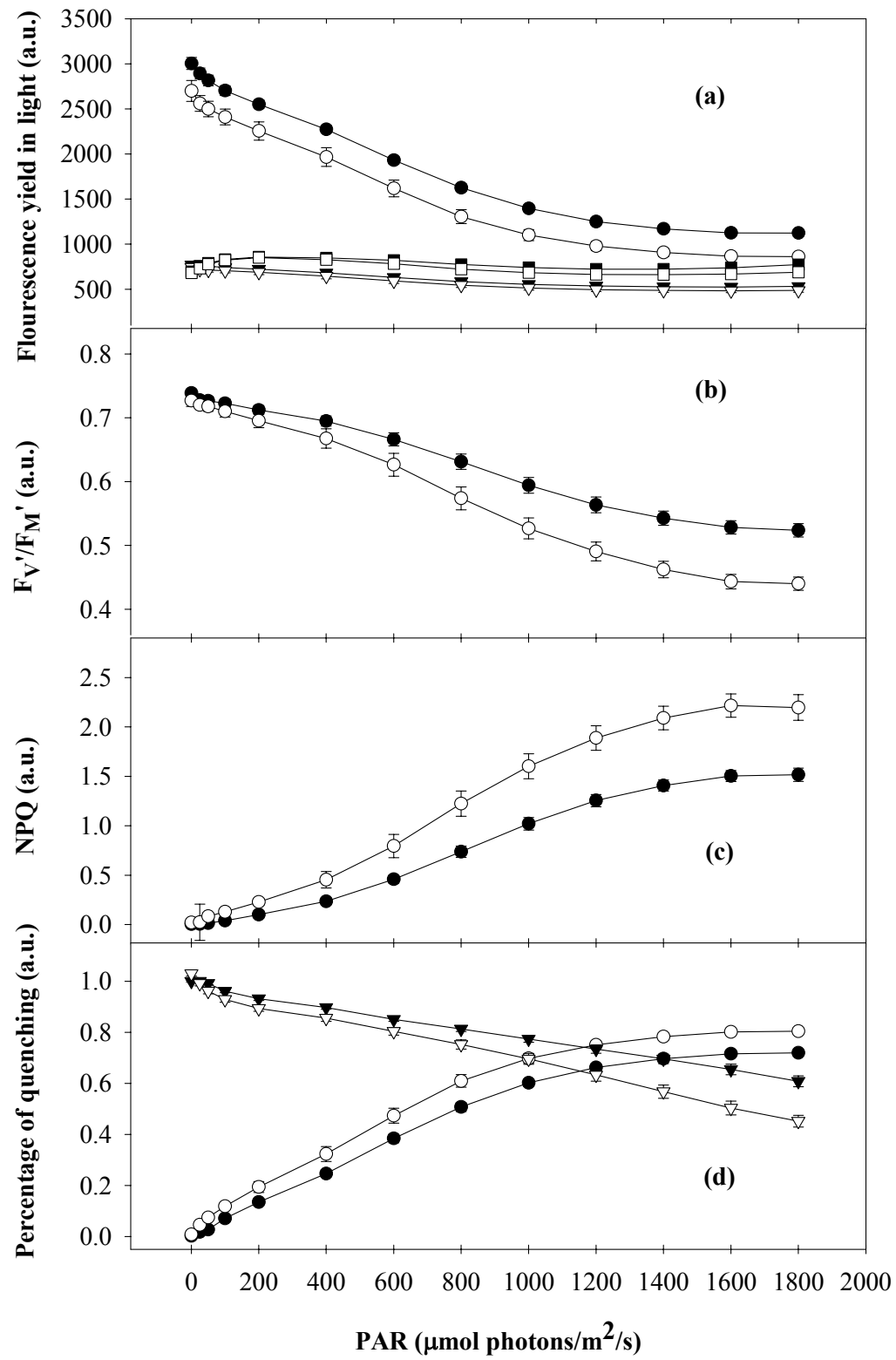


Fig 4

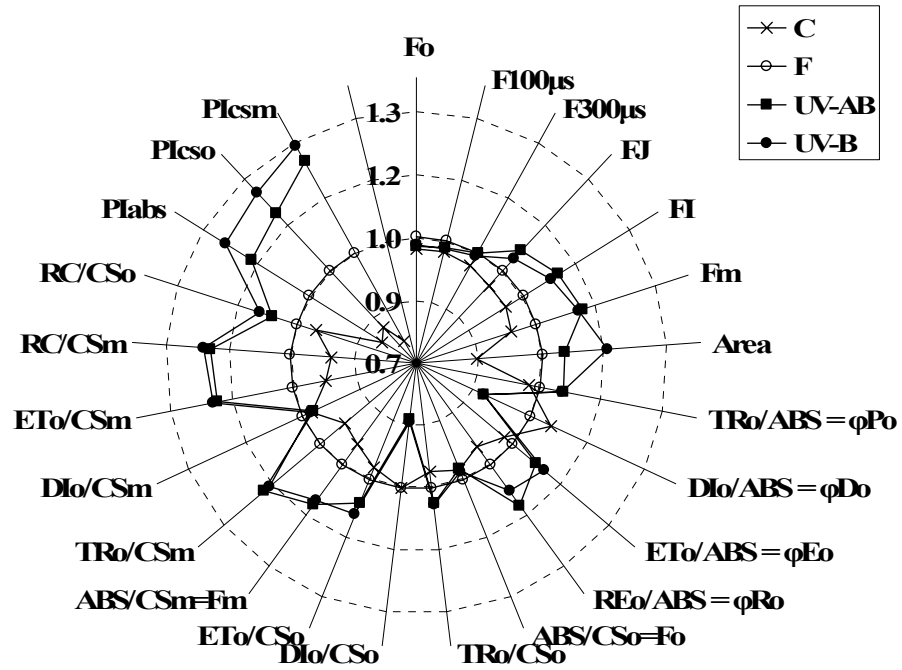


Table 1

	C	F	UVB	UVAB	Fvalue	ProbF
Measured parameters:						
F ₀	150 ± 2	154 ± 3	150 ± 2	149 ± 2	0.83	0.4766
F _{100μs}	161 ± 2	165 ± 3	162 ± 2	163 ± 2	0.71	0.5457
F _{300μs}	182 ± 3	189 ± 4	187 ± 3	189 ± 3	1.11	0.3447
F _J	294 ± 6 ^C	309 ± 7 ^{CB}	321 ± 6 ^B	329 ± 7 ^B	5.80	0.0007
F _I	399 ± 10 ^C	420 ± 9 ^B	452 ± 9 ^A	461 ± 10 ^A	9.41	<0.0001
F _m	503 ± 14 ^B	537 ± 13 ^B	594 ± 13 ^A	600 ± 14 ^A	11.60	<0.0001
t _{FM}	873 ± 8	887 ± 4	887 ± 4	869 ± 9	1.86	0.1351
Area	23693 ± 1014 ^C	28068 ± 1254 ^B	32367 ± 1141 ^A	29534 ± 1051 ^{AB}	8.08	<0.0001
Derived parameters:						
<i>Selected J-I-P-parameters</i>						
V _J	0.418 ± 0.008	0.420 ± 0.010	0.394 ± 0.009	0.408 ± 0.009	1.67	0.1734
V _I	0.716 ± 0.007 ^A	0.710 ± 0.009 ^{AB}	0.687 ± 0.006 ^B	0.700 ± 0.006 ^{AB}	3.33	0.0190
(dV/dt) ₀ = M ₀	0.388 ± 0.011	0.396 ± 0.017	0.356 ± 0.022	0.374 ± 0.014	1.13	0.3365
<i>Performance indexes</i>						
PI _{ABS}	3.01 ± 0.21 ^B	3.79 ± 0.34 ^B	4.68 ± 0.27 ^A	4.42 ± 0.27 ^A	7.39	<0.0001
PI _{CS₀}	441 ± 30 ^C	540 ± 43 ^B	679 ± 36 ^A	641 ± 35 ^A	8.79	<0.0001
PI _{CS_m}	1738 ± 156 ^C	2292 ± 247 ^B	2963 ± 201 ^A	2863 ± 202 ^A	7.82	<0.0001
<i>Phenomenological fluxes</i>						
TR _o /CS _m	354 ± 14 ^B	383 ± 13 ^B	443 ± 13 ^A	450 ± 14 ^A	12.24	<0.0001
DI _o /CS _m	150 ± 2	154 ± 3	150 ± 2	149 ± 2	0.83	0.4766
ET _o /CS _m	210 ± 9 ^B	228 ± 10 ^B	273 ± 9 ^A	270 ± 9 ^A	11.12	<0.0001
RC/CS _m	396 ± 18 ^B	438 ± 19 ^B	528 ± 18 ^A	521 ± 19 ^A	12.54	<0.0001
TR _o /CS _o	101 ± 2 ^A	105 ± 2 ^A	109 ± 2 ^B	109 ± 2 ^B	5.75	0.0007
DI _o /CS _o	48 ± 2 ^B	48 ± 2 ^B	41 ± 2 ^A	40 ± 2 ^A	4.56	0.0037
ET _o /CS _o	59 ± 1 ^B	61 ± 1 ^B	67 ± 1 ^A	65 ± 1 ^A	5.78	0.0007
RC/CS _o	112 ± 2 ^C	117 ± 2 ^B	128 ± 3 ^A	124 ± 3 ^A	8.57	<0.0001
<i>Specific fluxes</i>						
ABS/RC	1.44 ± 0.07	1.40 ± 0.06	1.27 ± 0.07	1.29 ± 0.05	1.75	0.1572
TR _o /RC	0.92 ± 0.01	0.92 ± 0.02	0.88 ± 0.02	0.90 ± 0.01	1.70	0.1660
DI _o /RC	0.53 ± 0.06	0.48 ± 0.05	0.39 ± 0.06	0.39 ± 0.04	1.54	0.2036
ET _o /RC	0.53 ± 0.01	0.52 ± 0.01	0.52 ± 0.01	0.52 ± 0.01	0.52	0.6662
<i>Quantum efficiencies</i>						
TR _o /ABS = φ _{Po}	0.677 ± 0.011 ^A	0.694 ± 0.011 ^A	0.732 ± 0.010 ^B	0.732 ± 0.010 ^B	7.18	<0.0001
DI _o /ABS = φ _{Do}	0.323 ± 0.011 ^A	0.306 ± 0.011 ^A	0.268 ± 0.010 ^B	0.267 ± 0.010 ^B	7.19	<0.0001
ET _o /ABS = φ _{Eo}	0.398 ± 0.009 ^B	0.409 ± 0.011 ^B	0.448 ± 0.010 ^A	0.437 ± 0.010 ^A	5.60	0.0009
RE _o /ABS = φ _{Ro}	0.196 ± 0.006 ^B	0.207 ± 0.008 ^B	0.223 ± 0.006 ^A	0.232 ± 0.006 ^A	6.33	0.0003
ET _o /TR _o = ψ _o	0.582 ± 0.008	0.580 ± 0.010	0.606 ± 0.009	0.592 ± 0.009	1.67	0.1733
RE _o /ET _o = δ̄ _o	0.481 ± 0.008 ^B	0.490 ± 0.009 ^B	0.517 ± 0.006 ^A	0.504 ± 0.006 ^{AB}	4.77	0.0028

Table 2

Leaf characteristics	Site	C	F	UVB	UV-AB	Fvalue	Prob
UV-B absorbing compounds	Fixed angle	0.91 ± 0.08	0.95 ± 0.06	0.85 ± 0.05	0.84 ± 0.06	1.21	0.3144
	Sloping	0.39 ± 0.06	0.35 ± 0.03	0.32 ± 0.05	0.24 ± 0.02	1.55	0.2577
	Level	0.42 ± 0.10	0.34 ± 0.06	0.29 ± 0.05	0.30 ± 0.07	2.09	0.1597
Carbon (%)	Fixed angle	45.67 ± 0.28	45.79 ± 0.27	45.29 ± 0.27	45.19 ± 0.25	0.91	0.4424
	Sloping	46.01 ± 0.37	45.61 ± 0.48	44.87 ± 0.31	45.07 ± 0.43	0.90	0.4945
	Level	46.27 ± 0.37	45.13 ± 0.28	45.56 ± 1.03	45.5 ± 0.52	1.65	0.2914
Nitrogen (%)	Fixed angle	2.19 ± 0.09	2.09 ± 0.12	2.13 ± 0.11	2.25 ± 0.10	0.46	0.7084
	Sloping	2.25 ± 0.18 ^{AB}	2.18 ± 0.05 ^{BC}	2.49 ± 0.17 ^{AB}	2.61 ± 0.10 ^A	4.82	0.0488
	Level	2.02 ± 0.05 ^{BC}	2.17 ± 0.13 ^{AB}	2.17 ± 0.07 ^{AB}	2.44 ± 0.22 ^A	8.70	0.0198
SLA (cm ² /g DW)	Fixed angle	0.089 ± 0.001	0.086 ± 0.001	0.087 ± 0.001	0.087 ± 0.001	1.31	0.2828
	Sloping	0.137 ± 0.021	0.211 ± 0.057	0.151 ± 0.016	0.298 ± 0.063	0.59	0.6324
	Level	0.633 ± 0.042	0.405 ± 0.087	0.514 ± 0.146	0.375 ± 0.112	0.14	0.9357
Maximal fluorescence, F _M	Fixed angle	0.744 ± 0.021 ^C	0.808 ± 0.018 ^B	0.866 ± 0.017 ^A	0.890 ± 0.017 ^A	12.35	<0.0001
	Sloping	0.872 ± 0.018 ^C	0.948 ± 0.012 ^B	1.017 ± 0.013 ^A	0.972 ± 0.015 ^{AB}	17.75	<0.0001
	Level	0.870 ± 0.020 ^{AB}	0.838 ± 0.016 ^B	0.957 ± 0.016 ^A	0.949 ± 0.019 ^A	10.22	<0.0001
Minimal fluorescence, F _O	Fixed angle	0.220 ± 0.003	0.222 ± 0.003	0.227 ± 0.003	0.224 ± 0.003	0.86	0.4598
	Sloping	0.273 ± 0.008 ^{AB}	0.294 ± 0.008 ^A	0.267 ± 0.006 ^B	0.250 ± 0.006 ^B	6.36	0.0003
	Level	0.250 ± 0.003 ^{AB}	0.249 ± 0.004 ^{AB}	0.238 ± 0.003 ^B	0.258 ± 0.005 ^A	4.07	0.0075
Maximum quantum yield, F _v /F _M	Fixed angle	0.677 ± 0.010 ^C	0.708 ± 0.008 ^B	0.726 ± 0.006 ^A	0.737 ± 0.008 ^A	10.61	<0.0001
	Sloping	0.680 ± 0.010 ^B	0.687 ± 0.009 ^B	0.735 ± 0.007 ^A	0.739 ± 0.007 ^A	13.79	<0.0001
	Level	0.703 ± 0.007 ^{BC}	0.697 ± 0.006 ^C	0.748 ± 0.005 ^A	0.724 ± 0.006 ^B	14.56	<0.0001
Steady state fluorescence, F _S '	Fixed angle	0.196 ± 0.004 ^C	0.207 ± 0.005 ^B	0.211 ± 0.004 ^B	0.218 ± 0.005 ^A	4.67	0.0032
	Sloping	0.213 ± 0.004	0.215 ± 0.004	0.215 ± 0.003	0.208 ± 0.003	0.81	0.4881
	Level	0.210 ± 0.004	0.217 ± 0.004	0.205 ± 0.004	0.213 ± 0.004	1.54	0.2042
Maximal fluorescence, F _M '	Fixed angle	0.434 ± 0.015 ^C	0.495 ± 0.016 ^B	0.532 ± 0.014 ^A	0.564 ± 0.014 ^A	14.34	<0.0001
	Sloping	0.434 ± 0.014 ^B	0.457 ± 0.012 ^{AB}	0.503 ± 0.015 ^A	0.495 ± 0.017 ^A	6.50	0.0004
	Level	0.426 ± 0.016 ^B	0.458 ± 0.017 ^{AB}	0.500 ± 0.019 ^A	0.488 ± 0.019 ^A	5.31	0.0014
Minimal fluorescence, F _O '	Fixed angle	0.159 ± 0.002 ^B	0.164 ± 0.002 ^{AB}	0.167 ± 0.003 ^A	0.169 ± 0.002 ^A	3.04	0.0288
	Sloping	0.171 ± 0.002 ^{AB}	0.168 ± 0.002 ^{AB}	0.173 ± 0.002 ^A	0.164 ± 0.002 ^B	3.14	0.0255
	Level	0.164 ± 0.003	0.165 ± 0.003	0.163 ± 0.003	0.164 ± 0.002	0.15	0.9273
Quantum yield, F _v '/F _M '	Fixed angle	0.633 ± 0.014 ^C	0.667 ± 0.014 ^B	0.685 ± 0.012 ^A	0.700 ± 0.012 ^A	8.59	<0.0001
	Sloping	0.778 ± 0.021 ^B	0.786 ± 0.019 ^B	0.842 ± 0.016 ^{AB}	0.812 ± 0.020 ^A	3.16	0.0247
	Level	0.777 ± 0.020 ^B	0.768 ± 0.025 ^B	0.842 ± 0.018 ^A	0.798 ± 0.021 ^{AB}	5.18	0.0017
NPQ = (F _M -F _M ')/F _M '	Fixed angle	0.80 ± 0.04 ^A	0.74 ± 0.05 ^A	0.70 ± 0.03 ^{AB}	0.65 ± 0.04 ^B	2.64	0.0488
	Sloping	1.16 ± 0.07	1.20 ± 0.06	1.18 ± 0.08	1.16 ± 0.08	0.10	0.9623
	Level	1.18 ± 0.07	0.98 ± 0.07	1.06 ± 0.07	1.09 ± 0.07	1.85	0.1377
qN = 1-(F _M '-F _O ')/(F _M -F _O)	Fixed angle	0.48 ± 0.02 ^A	0.45 ± 0.02 ^A	0.44 ± 0.01 ^A	0.41 ± 0.01 ^B	4.41	0.0045
	Sloping	0.57 ± 0.02	0.55 ± 0.02	0.56 ± 0.02	0.54 ± 0.02	0.54	0.6521
	Level	0.59 ± 0.02 ^A	0.51 ± 0.02 ^B	0.54 ± 0.02 ^{AB}	0.54 ± 0.02 ^{AB}	3.68	0.0127
qP = (F _M '-F _S ')/(F _M '-F _O ')	Fixed angle	0.84 ± 0.01	0.85 ± 0.01	0.86 ± 0.01	0.86 ± 0.01	1.10	0.3498
	Sloping	0.78 ± 0.02 ^B	0.80 ± 0.02 ^{AB}	0.84 ± 0.02 ^A	0.81 ± 0.02 ^{AB}	3.16	0.0247
	Level	0.78 ± 0.02 ^B	0.77 ± 0.03 ^B	0.84 ± 0.02 ^A	0.80 ± 0.02 ^{AB}	5.18	0.0017

Paper VIII

Ambient UV-radiation decreases PSII performance in spite of increased screening ability via UV-absorbing compounds in *Salix arctica* and *Vaccinium uliginosum* during six years of UV-exclusion in high arctic heaths.

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Abstract

We investigated the effects of ambient solar ultraviolet (UV) radiation on the wide spread dwarf shrub *Salix arctica* and *Vaccinium uliginosum* in a high arctic heath in Zackenberg, North-east Greenland. During six years UV-exclusion was conducted in the growing season by means of filters: 60% UV-B reduction, 90% UV-B +UV-A reduction, UV transparent filter control, and a control treatment without filter. Plant responses were evaluated by determination of leaf content of carbon, nitrogen and UV-B absorbing compounds and PSII performance parameters derived from recordings of chlorophyll-a fluorescence induction curves. The reduction of ambient UV-radiation resulted in significant stress release, i.e. higher F_v/F_M and PI in both species, and the concentration of UV-B absorbing compounds were reduced. The impact of ambient UV-B on F_v/F_M and PI are discussed in relation to the function of UV-B absorbing compounds. Further, the responses are discussed in relation to possible acclimation and impact on photosynthetic performance. It is concluded that ambient UV-B is an important stressor for dominant arctic heath plants. This may affect their performance in the future due to the possible increase in ambient UV-B radiation per se but also due to warming causing earlier start and extension of the growth season.

Keywords

Ozone depletion, long-term experiment, chlorophyll fluorescence, UV-B, heathland, Zackenberg

Introduction

Stratospheric ozone depletion is followed by an increase in the UV-B radiation (280 to 320 nm) in the biosphere (Webb 1997; Madronich et al., 1998). In the arctic region the UV-B irradiance level is currently considered to be near its maximum and the ozone column is estimated to recover towards the middle of the century, but the rate of ozone recovery is especially uncertain for the northern hemisphere (WMO, 2003). It has been argued that a possible increased cloudiness can counteract the effects of decreased ozone column thickness and thereby result in decreased UV-B radiation reaching the ground (Dorman and Woodin 2002). However, the predictions of cloudiness and cloud types are uncertain (Weathershead et al., 2005) and the increased amount of greenhouse gases in the atmosphere may increase the ozone destruction in the polar stratospheric clouds (WMO, 2003), which adds to the uncertainty of future UV-B load. High arctic plants are living in an extreme environment with a short growing season, low temperature, and often nutrient and water limitation. Environmental changes such as increased UV-B radiation which may cause additional stress (Caldwell et al., 1980; Bredahl et al., 2004; Albert et al., 2005). The relative increase in UV-B irradiance has been occurring most rapidly at high latitudes and also the absolute net depletion of ozone has been highest here, why the potential impact on the vegetation is expected to be high in this region (Björn et al., 1999; Paul, 2001). Few studies have addressed the impact of ambient UV-radiation on vegetation (Huskies et al., 2001; Day et al., 2001; Robson et al., 2003) and fewer in the Arctic (Gehrke et al., 1996; Gwynn-Jones and Johanson, 1996; Gwynn-Jones et al., 1997; Björn et al., 1999) although the need for further studies particularly in this region is widely recognized and motivated (Callaghan et al., 2004b; 2005).

A key target for UV-impact on plant performance is the photosynthetic machinery. This has been addressed in earlier studies in NE-Greenland (Bredahl et al., 2004; Albert et al., 2005; 2008). A manipulative approach with exclusion of large proportions of the natural UV-radiation by means of filters was adopted, thus avoiding the problems with spectral matching of ambient irradiation as experienced in many earlier UV-B supplementation experiments (Caldwell and Flint 1994; Searles et al., 2001). This enables the study of effects of reducing the present UV-B irradiance received by high arctic vegetation. We have previously in short term experiments shown that reduction of ambient UV-B resulted in increased F_v/F_m in *Salix arctica* and *Vaccinium uliginosum*, reduced stomatal conductance and internal CO₂-concentration in *Salix* (Bredahl et al., 2005) and decreased net photosynthesis in *Vaccinium* (Albert et al., 2008). These clear responses differ both in strength and direction from other similar studies on arctic plants (Callaghan et al., 2004c; Gwynn-Jones et al., 1997; Rozema et al., 2006; Phoenix et al., 2001) and long term investigations using UV-B exclusion are therefore urgently needed. In order to investigate several long term responses and possible acclimation responses we here report an analysis of six years of continuous measurements of chlorophyll-*a* fluorescence and UV-B absorbing compounds in leaves of the dominant dwarf shrubs *Salix arctica* and *Vaccinium uliginosum* exposed to ambient and reduced UV-B radiation in a high arctic heath in NE Greenland. We hypothesized that (1) ambient UV-B affects the physiological performance of the dwarf shrubs as (2) ambient UV-B increases the concentration of UV-B absorbing compounds, (3) that this increase in screening ability protects the underlying tissues from negative effects on PSII performance and (4) that a long term balanced response allow the plants to avoid negative effects on PSII.

Materials and methods

Experimental sites

The fieldwork was carried out in a high arctic heathland at Zackenberg Ecological Research Station, North East Greenland, (74°N; 21°E) throughout the growing season each year, ranging from early July to late August, during six years from 2001 to 2007. For logistic reasons the plots were left untreated in 2004. The plant species investigated were the widespread deciduous dwarf shrubs *Vaccinium uliginosum* L., *ssp. microphyllum* Lge. and *Salix arctica* Pall. (Böcher et al. 1978), which made up c. 60 and 20% of the vegetation cover, respectively. Two sites were established on south facing slopes, chosen to maximize the incoming amount of radiation and to make possible that the plots could benefit from uphill precipitation. Site 1 was marginally sloping (app. 5 degrees) while the inclination of site 2 was much steeper (app. 45 degrees); in the following referred to as ‘level site’ and ‘sloping site’, respectively. Both sites were dominated by *Vaccinium uliginosum* and *Salix arctica*. See also site description in Bredahl *et al.* (2004).

Experimental setup and treatments

The aim was to establish plots where parts of the UV spectrum in natural daylight were reduced, with ambient UV irradiance as reference. Reductions were achieved by filtering the solar radiation through two different filters, Mylar® (type D; DuPont Teijin Films, Wilmington, Delaware, USA) and Lexan® (RIAS, Roskilde, Denmark). In general the Mylar filter transmits $\lambda > 320$ nm (Cybulski and Peterjohn, 1999) and Lexan transmits $\lambda > 400$ nm (Bredahl *et al.* 2004). As control Teflon® filter (Fluoretek AB, Knivsta,

Sweden) was adopted. In general it transmits $\lambda > 280$ nm (Cybulski and Peterjohn, 1999). None of the filters had phytotoxic effects such as that reported for cellulose diacetate by Krizek and Mirecki (2004). The filters were mounted on 40x60 cm aluminum frames fixed approximately 5 cm above the mean vegetation height. At both sites the four treatments were replicated four times in a randomized block design.

Measurements in the experimental area with a broad-band cosine corrected UV-B sensor (UV-S-B-T, Kipp & Zonen B.V., Delft, The Netherlands) showed that the plant canopy under Teflon, Mylar and Lexan, respectively, were exposed to approximately 91%, 39% and 17% of the clear sky UV-B irradiance and to 97%, 90% and 91% of the photosynthetically active radiation (PAR), slightly depending on the exposure angle to the sun (Bredahl et al., 2004). The 17% UV-B measured under Lexan and a fraction of the clear sky UV-B irradiance in the other treatments, are due to diffuse and reflected radiation reaching the UV-B sensor from the open sides not covered with filters (Bredahl et al. 2004). Teflon is referred to as “F”, Mylar as “UV-B” and Lexan as “UV-AB”.

A fourth treatment was an open control, C, without any filter. Filters were replaced with new ones every year. Only minor and insignificant filter ageing were detected in the PAR (400-700nm) and UV-B (280-320nm) band when transmittance measurements were conducted on filters before and after 2 months exposure to ambient UV (pers. com.; Hanson S.G., Dam-Hansen C., Optics and Plasma Research Department, Risø-DTU, DK).

Microclimate

Two identical data loggers (CR 10X, Campbell Scientific, Ltd., Loughborough, UK) continuously recorded the following climatic parameters on both sites during 2001-2003: Incoming photosynthetically active radiation, PAR, was measured perpendicular to the slope with GaAsP photodiodes (Pontailler, 1990); Soil moisture in each of the four blocks at the two sites in 0-30 cm depth were measured with CS615 Water Content Reflectometers (Campbell Scientific); In all 2x16 plots canopy air temperature and soil temperature in 3 cm depth were measured with epoxy (air) or rubber (soil) coated thermocouples connected to an AM25T Solid State Multiplexer (Campbell Scientific). Additional irradiance data was obtained from the meteorological station at Zackenberg about 1 km from the sites during each summer 2000-2007, except in some months before July 28th 2001 where no data were available due to a malfunctioning sensor (data provided by the Zackenberg Database, <http://zdb.dmu.dk>). Each hour the current UV-B flux was recorded by a horizontally mounted erythema-weighted UV-Biometer in 2m height (501A, Solar Light Co. Inc., Philadelphia, PA, USA). PAR were measured with a quantum sensor (Licor 190 SA, LI-COR Environmental, Nebraska, USA) in $\mu\text{mol photons/m}^2/\text{s}$ each hour.

Leaf content of carbon, nitrogen and UV-B absorbing compounds

Each year, leaves from *Salix arctica* and *Vaccinium uliginosum* were harvested in the end of each growing season in early to mid august except for *Salix* in 2007. Photographs of the leaves beside a brick of known area allowed for leaf area determination. Afterwards leaves were dried at 80°C for 48-62 hours, dry weight determined and then ground. Total

soluble UV- absorbing compounds were extracted by a three step procedure: 1) 1 mg of the grinded leaves were heated (3 min, 60°C) in 5ml methanol; 2) 10 min shaking after adding 5ml HCl-H₂O-methanol (1:20:79); 3) centrifugation (5000 rpm, 10 min) and tenfold dilution of the supernatant in HCl-H₂O-methanol (1:20:179) (Caldwell 1968). After additional 10 min shaking the UV-absorption was measured with a spectrophotometer (Hitachi, U-2010, Tokyo, Japan) in the interval 280-315 nm by 0.2 increments. The area below the absorption curve was calculated as the sum of all absorptions in the scanned range. Total leaf carbon and nitrogen were determined on a LECO truespecTM CN elemental determinator (Leco Corporation, Michigan, USA).

Chlorophyll-a fluorescence

Dark adaptation clips were randomly mounted on healthy leaves of *Salix* (5 clips, on separate shoots) and on top canopy leaves of *Vaccinium* (10 clips, on separate shoots) for 35-40 min in each plot. As the *Vaccinium* leaves were very small they had to be detached before clip mounting. Darkening of leaves ensured that all PhotoSystem II's (PSII's) were in the dark adapted state with open Reaction Centers (Krause and Weiss 1992). Then chlorophyll-a fluorescence transients were recorded and digitized with a portable Handy PEA (Hansatech Instruments, Ltd. King's Lynn Norfolk, UK) with a 12-bit resolution from 10 µs to 1s and a time resolution of 10 µs for the first 200 data points (Strasser and Strasser 1995). The excitation light intensity was 600 Watt/m² from an array of six red light emitting diodes with a peak wavelength at 650 nm focused on the leaf surface in the clips on a spot of 4 mm in diameter. The measurements were always done in the same order, alternating between different treatments in each block one to two times a week. Prior to variance analysis, extreme values were excluded by objective criteria:

$0.01 < PI_{Abs} < 10$ and $0.4 < F_V/F_M < 0.85$. This decreased the number of measurements from 12240 to 11972 for *Vaccinium* and 14310 to 14247 for *Salix*. In agreement with earlier studies by Bredahl et al. (2004) and Albert et al. (2005), July was the peak growth season. In order to limit comparisons of treatment, species and years to a fixed time period, the July data were further extracted reducing to 11355 measurements for *Vaccinium* and to 12447 for *Salix*.

Analysis of fluorescence

From the fluorescence transient, the measured parameters F_0 , F_M , tF_M , Area, $F_{50\mu s}$, $F_{100\mu s}$, $F_{300\mu s}$, F_J , F_I were used to calculate and derive a range of parameters according to Strasser, Tsimilli-Michael and Srivastava (2000; 2004). The so-called JIP-test parameterization (Strasser et al., 2004) provides information of the stepwise flow of energy through PSII at different levels: 1) specific fluxes per reaction centre (RC), absorption (ABS/RC), trapping (TR_0/RC), dissipation (DI_0/RC) and electron transport (ET_0/RC); and 2) phenomenological fluxes per excited sample cross section (CS), absorption (ABS/CS), trapping (TR_0/CS), dissipation (DI_0/CS) and electron transport (ET_0/CS). By assuming that F_M were a reasonable measure of the absorption energy flux per excited cross-section (ABS/CS) of a particular leaf sample in the dark adapted state, phenomenological fluxes were estimated from F_M . The specific and phenomenological fluxes are interrelated by the quantum efficiencies, which are 1) the maximum quantum yield of primary photochemistry (F_V/F_M) which in this terminology is equal to the efficiency by which an absorbed photon will be trapped by the PSII RC with the resultant reduction of Q_A to Q_A^- (TR_0/ABS) and 2) the efficiency by which a trapped exciton,

having triggered the reduction of Q_A to Q_A^- can move an electron further than Q_A^- into the intersystem electron transport chain (ET_0/TR_0). Integrative parameters, so-called performance indexes reflecting performance of the overall energy flow processing are 1) based on quantum efficiencies and hereby related to the situation of assuming equal absorption (PI_{ABS}) and 2) based on the phenomenological fluxes related to the per leaf cross-section level (PI_{CS_0} and PI_{CS_M}). Estimation of the density of active PSII RC per CS (RS/CS_0 and RC/CS_M) is also possible. For a full summary of parameters, formulae and definition of symbols see Strasser et al. (2004) and tables in Albert et al. (2005).

Statistical analysis

Statistical analyses were conducted using the GLM procedure (SAS Institute, 2002). Levene's test was used to test for homogeneity of variance. Where necessary, parameters were transformed in order to meet the assumptions of ANOVA. In cases of significant treatment effects these analyses were followed by tests of treatment differences using Tukey's test. All values presented here are non-transformed. Differences are considered at the $P < 0.05$ level. Fluorescence data on *Salix* were tested with repeated measurement ANOVA's, with treatment and as factors and day repeated within each plot. This was done to take into account that the leaves were often sampled and measured on the same experimental unit (due to few occurring plant specimens in the plot) during the three succeeding years. The contrast option was used to test parameter levels between years. Because sampling of leaves for measurements were completely random within each plot for *Vaccinium*, a general linear model with treatment and year as factors were used.

Treatment effects on species were tested by joining data from both sites. When not otherwise noted the statistical analysis includes all treatments.

Results

Irradiance and microclimate

Irradiance of mean daily PAR in the horizontal plane did not differ significantly between the ‘level’ and ‘sloping’ site, but the PAR irradiance received from south by the sloping site was significantly higher than for the level site during all years with available data (2001, $p < 0.001$; 2002, $p < 0.002$; 2003, $p < 0.001$). Comparing the levels of irradiance between years showed average PAR level in July to be 424, 431, 464, 424, 550 and 531 $\mu\text{mol photons/m}^2/\text{s}$ in 2002-2007. The monthly accumulated erythremal weighted UV-B fluxes (MED/month) in July at the meteorological station in Zackenberg were 12.7, 12.5, 15.8, 13.3, 20.8, 17.9 MED/month on 2002-2007, respectively (Table 1). During these years the UV-B fluxes showed May and July fluxes were about the same level within every year in spite of considerable variability between years (Table 1). During all individual years June had both the highest maximum and the highest accumulated UV-dose, thereafter the monthly accumulated fluxes decreased in all years (Table 1).

Air temperature measured at the sites were 6.1°C, 6.2°C and 7.6°C in 2001, 2002 and 2003 across season (May through August) and sites, with 2003 as the warmest ($p < 0.0001$). The monthly mean temperatures of 7.7°C and 6.6°C in July and August 2003 were the highest during the years 1996-2003. In the following years July mean air temperatures were 7.5°C, 6.9°C and 6.6°C, respectively (Klitgaard et al., 2007). The

maximal temperature record 22.8°C since establishment of the meteorological station in 1995 was recorded 21. July 2006 (Klitgaard et al., 2007).

Air and soil temperatures fluctuated simultaneously in all treatments during all years, although the fluctuations in soil temperature were dampened as compared to air temperature. Treatment specific air temperature differences were not observed in 2001. In 2002 both the air and soil temperature were 0.3°C -0.5°C higher in the filtered treatments compared to the open control ($p < 0.0001$). In 2003, the mean air temperature level was 6.4°C in the control, the UV-B and UV-AB treatments were intermediate at 6.8°C and the F treatment was highest at 7.1°C. The mean soil temperature was lowest in the control with 4.8°C, the UV-B and UV-AB treatments were intermediate at 5.5°C and the F treatment were highest at 5.8°C ($p < 0.0001$). Soil water content in 6 cm depth at the 'level site' was around 0.45 m³/m³ as compared to 0.30-0.45 m³/m³ on the 'sloping site' (Bredahl et al., 2004). Generally, soil water content in 6 cm and 30 cm dept were slightly decreasing through the season, most pronounced in 2003 (data not shown). There was no difference between treatments in soil moisture in 2001 (Bredahl et al., 2004) or in 2003 (Riinnan et al., 2005).

Chlorophyll-a fluorescence

Response of Vaccinium uliginosum

The maximum quantum yield, F_v/F_m , across all filtered treatments was higher in 2001, 2002 and 2007, intermediate in 2003 and 2006 and low in 2005 ($p < 0.0012$). The values of the performance index based on equal absorption, PI_{Abs} , were high in 2002, 2005 and 2006, intermediate in 2003 and 2007 and lowest in 2001 ($p < 0.003$).

There was a clear seasonal variation in F_v/F_m and particular in PI_{Abs} during all years, identifying July as the period where peak values were recorded (data not shown).

Maximum quantum yield, F_v/F_m , were significantly higher during each year in July in the order UV-AB > UV-B > F > C (all $p < 0.0001$, Figure 1). Across all years the same order were found, with the UV-AB and UV-B treatments as significantly higher than F and C ($p < 0.0001$, Figure 1). In principle, this is the net result of either higher maximal fluorescence intensity, F_m , and/or higher variable fluorescence, $F_v (= F_m - F_o)$. The changes in F_v are due to either lower values of the initial fluorescence intensity, F_o , and/or higher maximal fluorescence intensity, F_m . Small increases in F_o were significant in some years, however F_m were strongly affected in most years (Figure 1).

The values of the derived performance indexes based on 1) equal absorption, PI_{Abs} , and 2) leaf absorption assuming proportional to F_m , PI_{CSm} , were higher in the order UV-AB > UV-B > F > C during all years (Figure 1). The performance indexes in UV-AB and UV-B treatments were significantly higher than in F ($p < 0.0001$) and (except in 2002) in C ($p < 0.0001$, Figure 1). Across all years the PI_{Abs} and PI_{CSm} in UV-AB and UV-B were highest and significantly different from F and C ($p < 0.0001$, Figure 3).

The relation between the phenomenological fluxes derived from F_m resulted in a higher electron transport per cross section of the leaf sample, ET_m/CS_m , in following decreasing order over all years: UV-AB > UV-B > F > C, with UV-AB and UV-B significantly different from each other and from F and C ($p < 0.0001$, Figure 1, 3). Potentially, this response was mediated by either higher energy trapping flux per cross section of the leaf, TR_m/CS_m , and/ or a lower dissipation of excess energy per cross section of the leaf, DI_m/CS_m . In most years the TR_m/CS_m were significantly higher in the UV-AB and UV-B

treatments compared to C and F, and the DI_m/CS_m in UV-AB and UV-B was significantly lower or leveled the F and C treatments (Figure 1, 3).

Density of active PSII reaction centre per cross section of the leaf, RC/CS_M , were higher during all years in the following order $UV-AB > UV-B > F > C$, except in 2002. The RC/CS_M were significantly higher in treatment UV-AB and UV-B compared to F and C in all years (all $p < 0.0001$, Figure 1), except in 2002 where no significant difference between treatments were found.

Response of Salix arctica

Over the years, the F_v/F_m was about the same level in 2001, 2002 and 2003, and significantly lower than in 2006 and 2007 ($p = 0.0037$). For the performance index PI_{Abs} the pattern were the same ($p = 0.0035$).

Maximum quantum yield, F_v/F_m , was higher in the UV-AB and UV-B treatment in all years, but only significantly in 2002, 2003 and 2007 (all $p < 0.0001$, Figure 2). Over all years the values of F_v/F_m were decreasing in the order $UV-AB > UV-B > C > F$, and the UV-AB and UV-B had significantly highest F_v/F_M ($p < 0.0001$, Figure 3), while the other treatments did not differ significantly.

Initial fluorescence values, F_o , were only slightly affected in most years (Figure 2), but decreased significantly across years in UV-AB and UV-B versus F and C ($p < 0.0001$, Figure 1), whereas the maximal fluorescence intensity, F_m , were more strongly affected and increased significantly over the years in UV-AB and UV-B versus F and C ($p < 0.0001$, Figure 2, 3).

In all individual years, except 2001, the values of PI_{Abs} and PI_{CSm} decreased in the order $UV-AB > UV-B > F > C$, with the UV -AB and UV-B treatment significantly different from F and C (all $p < 0.0001$, Figure 2). Across years same significant order were found ($p < 0.0001$, Figure 1).

The phenomenological fluxes TR_m/CS_m and in particular ET_m/CS_m were increased over the years ($p = 0.002$, $p < 0.0001$), whereas DI_m/CS_m were unaffected (Figure 3).

RC/CS_m values were higher in the UV-AB and UV-B in all years (Figure 2), but only significant across years ($p < 0.0001$, Figure 3).

Leaf content of carbon, nitrogen and UV-B absorbing compounds

The concentration of carbon and nitrogen per leaf dry weight did not vary between year, treatment or species. Hence the mean \pm standard error was $2.15 \pm 0.12\%$ N and $45.47 \pm 0.47\%$ C, respectively. Specific leaf area did not differ between treatments in any years. The means varied from 0.099 to 0.385 cm^2/mg in *Salix* and from 133 to 312 cm^2/mg in *Vaccinium* between years. On average it was 25% higher in *Vaccinium* compared to *Salix* across years. The treatment averages across all years, here presented as values in percent of treatment F, decreased in following order $C > F > UV-B > UV-AB$ for both species: *Salix* 114, 100, 94 and 88%; *Vaccinium* 103, 100, 92, 87 %. In both species the UV-B and UV-AB differed significantly from F and for *Salix* F also from C (both $p < 0.0001$).

For *Salix* there was an overall significant effect of treatment in year 2001, 2003 and 2006 ($p < 0.0365$, $p < 0.0001$, $p < 0.0001$), but no effect in year 2002 and 2005 ($p < 0.200$, $p < 0.260$). Inspection of the Tukey test for *Salix* in 2001 revealed a significant difference

between the UV-AB treatment and C. In 2003 there was a significant difference between C and filter treatments and the UV-AB was different from the other filter treatments (Table 4). Excluding the C treatment did not change the pattern of significance (data not shown). Only in 2006 there was a significant stepwise difference from C to F and to UV-B and UV-AB (Table 2)

For *Vaccinium* there was an overall effect of treatment in 2002, 2006 and 2007 ($p < 0.0005$, $p < 0.0001$, $p < 0.0001$), but none in 2001, 2003 and 2005 ($p < 0.2674$, $p < 0.1038$ and $p < 0.7119$). The Tukey test reveals a significant difference between the filter and open control, F and C, versus UV-B and UV-AB, in 2002, 2006 and 2007. When excluding the control treatment, C, the UV-AB and UV-B significantly differed from F, in 2002, 2006, 2007, while UV-B values from 2003 and 2005 were intermediate and not significant, although the UV-AB became significant different from F ($p < 0.0005$, $p < 0.0211$, data not shown).

Discussion

Experiments with supplemental UV-radiation (e.g. Paul, 2001; Björn et al., 1999; see also review by Flint et al., 2003) has led to the view that the impact of increased UV-radiation be may small compared to e.g. nutrient addition or warming (Caldwell et al., 2004c), and also that sub-arctic plants have some resilience to increased UV-radiation (Callaghan et al., 2004c). Some studies have evaluated plant responses to ambient UV- radiation (e.g. Robson et al., 2003; Day et al., 2001; Paul and Gwynn-Jones, 2003). Thus some of the identified plant responses to ambient UV-radiation are changes in leaf secondary chemistry characteristics (e.g. Searles et al., 2001; Albert et al., 2005), decrease in PSII

activity (e.g. Albert et al., 2005; Xiong and Day 2001) and photosynthetic parameters (e.g. Albert et al., 2008; Xiong and Day 2001). The present investigation was aiming on a first synthesis of the responses at two levels: The impact of naturally occurring UV-radiation doses on measures of photosynthetic performance/plant stress and on UV-B absorbing compounds as a measure of UV-screening capacity.

UV-B absorbing compounds

Accumulation of UV-B absorbing compounds are believed to provide a protective screen in the epidermis against UV-B radiation (Bornmann et al., 1997; Tevini and Teramura, 1989; Shehan, 1996; Meijkamp, 2001). Hence, as demonstrated by a meta analysis of 62 outdoor experiments, the accumulation of UV-B absorbing compounds seems to be the most consistent effect of enhanced UV-B on plants (Searles et al., 2001). This effect is also observed in some UV-B exclusion studies when comparing ambient UV-B to filter control (Ruhland and Day, 2000; Xiong and Day, 2001; Albert et al., 2005), although Phoenix et al., (2001) reported decreased content of UV-B absorbing compounds in ambient UV-B compared to filter control.

In the present study, a higher leaf content of UV-absorbing compounds was found for both *Salix* and *Vaccinium* across all years in the F and to some degree also in C treatments compared to UV-B and UV-AB. Interestingly the treatment differences, F vs UV-B and UV-AB, in UV-B absorbing compounds were the largest for both species in 2006, the year with the highest UV-B dose and smallest and insignificant in the year with smallest UV-B dose, 2005. These findings clearly indicate that the applied manipulative UV-screening is of a magnitude that controls the concentration of UV-B absorbing

compounds in both species and that the level to some degree is mediated by the current year ambient UV-B dose in deciduous plant species.

The species differed in response with *Vaccinium* showing a 25% higher content of UV-absorbing compounds compared to *Salix*. This indicates that *Vaccinium* is more UV-sensitive and/or has higher ability to synthesize UV-absorbing compounds than *Salix*. However, differences in levels of UV-B absorbing compounds between species could be due to other factors than UV-radiation i.e. indirect effects on plant nutrient status or the result of tradeoffs. For instance, the supply of carbon and nutrients, or herbivore pressure could influence the concentration of secondary metabolites (Hansen et al., 2006).

On the other hand, if the function as a screen against damaging UV-radiation is the most important function of UV-absorbing compounds, increased content should result in a stress release on photosynthetic performance. If so, this may directly be detected by i.e. measures of light energy to and electron processing through, the photosynthetic apparatus. Either ways, both seasonal and yearly variability in ambient UV-doses may also potentially affect the response, why significant plant responses are not necessarily to be expected every year. This leads to the hypothesis that since the content of UV-B absorbing compounds is higher in *Vaccinium* as compared to *Salix*, a lower impact of UV-radiation on PSII performance might be expected in *Vaccinium*.

PSII performance

Fluorescence transients are rich in information and the interpretation of JIP-test parameters extends the description the quantitative behavior of the PSII to much more than the only often reported parameter F_v/F_m .

Both species had a higher maximum quantum yield, F_v/F_m , in UV reduced treatments during all years, which clearly indicates that ambient UV-radiation is a stress factor affecting PSII activity. This appeared to be the result of combinations of either higher F_m and/or lower F_o , leading to higher $F_v (= F_m - F_o)$ in UV-reduced treatments for both species. In comparison, both higher F_m and lower F_o were observed on *Salix* leaves receiving homogenized doses of radiation in (Albert et al., 2005). Thus mechanisms affecting both F_o and F_m are probable.

PI-indexes (PI_{Abs} and PI_{CSm}) were, in parallel with F_v/F_m , higher in UV-reduced treatments in both species during all years, which clearly strengthens that ambient UV-radiation is a stress factor affecting PSII activity. The observed patterns in F_m do in combination with higher PI_{Abs} add in favor for even higher indices of PI_{CSm} . The PI_{Abs} is an index summing up the combined response of key PSII energy processes quantified by the so-called quantum efficiencies. Further, PI_{Abs} is mathematical ‘clean’ by assuming that leaves have the same absorption of light (Strasser et al., 2004). A decreased PI_{Abs} therefore gives biological sense as a measure of the effectiveness of the overall energy processing through the photosynthetic apparatus.

The behavior of the phenomenological fluxes (TR_m/CS_m , DI_mCS_m , ET_m/CS_m and RC/CS_m) in this study were closely in accordance with the pattern previously found for *Salix* after one year of treatment, see the detailed discussion of parameter relations and interpretation given by Albert et al., (2005). There was a significantly higher leaf content of chlorophyll a per fresh weight in UV-B screened treatments versus filtered and open control (data from in 2005 and 2006, unpublished). This adds to the probability of higher absorption energy flux in UV-B screened treatments in line with the above interpretation,

and it also supports a resulting higher potential for electron processing through the photosynthetic apparatus.

Possibly the decreased ET_m/CS_m observed here for *Vaccinium* and *Salix* may potentially be directly related to decreased photosynthesis under the assumption that ET_m/CS_m indeed is proportional to the actual electron flow to the Calvin Cycle, see discussion below. Actually a decreased net photosynthesis (and Performance indexes) was found in canopies of *Vaccinium* exposed to ambient UV-B on a nearby site (Albert *et al.* 2008). Therefore it cannot be ruled out that the decreased PI_{Abs} and F_v/F_m in ambient UV-B may indirectly point to potential effects further downstream for both species.

Are high arctic *Vaccinium* and *Salix* sufficiently acclimated to ambient UV-radiation?

Higher concentration of UV-absorbing compounds in parallel to higher ambient UV-radiation doses may indicate a plant response focused to screen off UV-radiation. Between treatments this response was seen when pooling data from all years and for *Vaccinium* in most individual years. Despite this response, neither *Vaccinium* nor *Salix* could avoid the impact of ambient UV-radiation on the photosynthetic apparatus across years. In fact, due to higher concentration of UV-absorbing compounds in *Vaccinium* was expected to screen off UV-B to a higher degree than *Salix*, and to show less impact on the photosynthetic apparatus compared to *Salix*. However, no species specific differences in fluorescence response in fx. the PI or F_v/F_m was seen (Figure 3). Nor did the differences in F_v/F_m , and for *Vaccinium* also PI , in UV-B screened versus the filtered control differ between the years 2005 and 2006 with the minimum and maximum average UV-B dose,

respectively. This clearly demonstrates that despite a UV-radiation avoidance response the investigated plants are actually stressed in ambient UV-radiation. Further, this also demonstrates that plant sensitivity to ambient UV-B can not be deduced from impact on the levels of UV-radiation absorptive compounds alone.

Plants may be speculated to show long term responses to minimizing the effects of UV-radiation on key parameters related to the photosynthetic performance. If occurring, this acclimation response should primarily minimize the differences between filtered treatments. However the repeated observed pattern in our study of effects on PSII performance every year in *Vaccinium* and *Salix* did not support this notion.

We believe that the varying leaf angles and thereby different irradiance doses received by the leaves, lead to the high variation and to some degree extreme fluorescence values which were excluded by objective criteria. This is supported by the findings of clear reductions of F_v/F_m and PI's and negative impact on most other parameters in ambient UV-B, when the natural irradiance doses were homogenized (Albert et al., 2005). Because a high number of leaf samples were measured each year, relatively small changes in measured parameters may become significant. Evaluation of whether parameter differences actually are small or large must bear in mind the nature of the parameter investigated. Although parameters from the chlorophyll-a fluorescence induction curve are widely accepted and used as indicators of plant stress (Krüger et al., 1997; Clark et al., 2000; Strasser et al., 2004; Bussotti, 2004) they are biophysical and measured in relative units.

From a theoretical point of view it could be questioned to which degree chlorophyll-a fluorescence induction parameters (JIP-test) are linked to Calvin Cycle

activity and thereby primary production. By assuming i.e. linear electron transport, some biochemical experimental studies (Force et al., 2003; Heredia and De Las Rivas, 2003; van Heerden et al., 2003) and numerical simulations (Stirbet et al., 1998) do support this. However, an obstacle with the induction curves is that they are measured on dark adapted samples, which then reflects the behavior of PSII under ‘relaxed’ conditions i.e. at their maximal performance. Therefore, if possible, it would be preferable to do the JIP-test on illuminated leaves. A protocol allowing for light acclimation to different light levels is one step. Next is to apply far-red illumination to determine initial fluorescence in light. Such a protocol could provide all traditional fluorescence parameters i.e. maximal, minimal and steady state fluorescence yield in light for calculation of quantum yield and quenching parameters in supplement to JIP-test parameters.

We argue that although the JIP-test gives a detailed description of the proportions of actual energy fluxes, only if PSII really are the primary target caused by the investigated stressor, the chlorophyll-a fluorescence induction parameters are likely to translate into changes in Calvin Cycle activity further downstream. Hence, the interpretation of negative impacts on chlorophyll-a fluorescence induction parameters (JIP-test) are, in a strict sense, limited to stress solely affecting PSII processes in the photosynthetic apparatus. The parallel increase in e.g. F_v/F_m , PI_{Abs} and net photosynthesis in reduced UV-B for *Vaccinium* canopies clearly pointed to the potential of decreased PSII activity (Albert et al., 2008), although direct effects on Calvin Cycle were not ruled out. The clear detection of negative impacts on chlorophyll-a fluorescence induction parameters across the great variation of natural leaf angle in this study clearly points to ambient UV-radiation as an important stressor in high arctic plants. Even higher impact is

deduced by increasing natural UV-radiation doses, as demonstrated in practice by maximizing the ambient UV-B level by Albert et al., (2005). This may not only be mediated by changes in ozone layer thickness and cloud cover alone, since an earlier onset of spring will expose vegetation to the higher UV-B spring fluxes in June.

Conclusion

The investigated plants are every year responding significantly to the current UV-radiation: Despite plants responded with increased concentration of UV-B absorbing compounds, negative effects on PSII performance can not be avoided. In this perspective ambient UV-radiation are an important stressor for high arctic plants and increased impact are probable in years with early start of growing season. This point to the importance of long-term monitoring of effects of ambient UV-B on the photosynthetic performance, and to continued investigations focused to synthesize plant responses on across scales.

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References

Albert K.R., Mikkelsen T.N., Ro-Poulsen H., 2005. Effects of ambient versus reduced UV-B radiation on high arctic *Salix arctica* assessed by measurements and calculations of chlorophyll-*a* fluorescence parameters from fluorescence transients. *Physiologia Plantarum* 124, 208-226.

Albert K.R., Mikkelsen T.N., Ro-Poulsen H., 2008. Ambient UV-B radiation decreases photosynthesis in high arctic *Vaccinium uliginosum*. *Physiologia Plantarum* 133, 199-210.

Bornman J.F., Reuber S., Cen Y.-P., Weissenböck G., 1997. Ultraviolet radiation as a stress factor and the role of protective pigments. In: *Plant and UV-B: Responses to environmental change* (ed P. Lunsden), pp 157-170. Society for Experimental Biology seminar series: 64. Cambridge University Press, Cambridge, United Kingdom, ISBN 0-521-57222-3.

Björn L.O., Callaghan T.V., Gehrke C., Gwynn-Jones D., Lee J.A., Johanson U., Sonesson M., Buck N.D., 1999. Effects of ozone depletion and increased ultraviolet-B radiation on northern vegetation. *Polar Research* 18, 331-337.

Böcher T.W., Fredskild B., Holmen K., Jacobsen K., 1978. Grønlands Flora. P. Haase & søns forlag, Copenhagen, Denmark, ISBN 87-559-0385-1.

Bredahl L., Ro-Poulsen H., Mikkelsen T.N., 2004. Reduction of the Ambient UV-B Radiation in the High Arctic increases F_v/F_m in *Salix arctica* and *Vaccinium uliginosum* and reduces Stomatal Conductance and internal CO₂ Concentration in *Salix arctica*. Arctic, Antarctic and Alpine Research 36, 364-369.

Bussotti F., 2004. Assesment of stress conditions in *Quercus ilex* L. leaves by O-J-I-P chlorophyll alpha analysis. Plant Biosystems 138, 101-109.

Caldwell M.M., 1968. Solar ultraviolet radiation as an ecological factor for alpine plants. Ecological Monographs 39, 243-268.

Caldwell M.M., Robberecht R., Billings W.D., 1980. A steep latitudinal gradient of solar ultraviolet-B radiation in the arctic-alpine life zone. Ecology 61, 600-611.

Caldwell M.M. and Flint S.D., 1994. Stratospheric ozone reduction, solar UV-B radiation and terrestrial ecosystems. Climatic Change 28, 375-394.

Callaghan T.V., Björn L.O., Chernov Y., Chapin T., Christiansen T.R., Huntley B., Ims R.A., Johansson M., Jolly D., Jonasson S., Matveyeva N., Panikov N., Oechel W. and Shaver G., 2004a. Uncertainties and recommendations. Ambio 33, 474-479.

Callaghan T.V., Björn L.O., Chernov Y., Chapin T., Christiansen T.R., Huntley B., Ims R.A., Johansson M., Jolly D., Jonasson S., Matveyeva N., Panikov N., Oechel W. and Shaver G., 2004b. Rationale, concepts and approach to the assessment. *Ambio* 33, 393-377.

Callaghan T.V., Björn L.O., Chernov Y., Chapin T., Christensen T.R., Huntley B., Ims R.A., Johansson M., Jolly D., Jonasson S., Matveyeva N., Panikov N., Oechel W., Shaver G., Elster J., Jónsdóttir I.S., Laine K., Taulavuori K., Taulavuori E., Zöckler C., 2004c. Responses to projected changes in climate and UV-B at the species level. *Ambio* 33, 418-435.

Callaghan T.V., Björn L.O., Chapin T., Chernov Y., Christensen T.R., Huntley B., Ims R.A., Johansson M., Riedlinger Jolly D., Jonasson S., Matveyeva N., Oechel W., Panikov N., Shaver G., 2005. In: ACIA, Arctic Climate Impact Assessment (eds Symon C., Arris L. and Heal B.), pp. 243–352, Cambridge University Press, New York.

Clark A.J., Landolt W., Bucher J.B., Strasser R.J., 2000. How wind affects the photosynthetic performance of trees: Quantified with chlorophyll-a fluorescence and open-top chambers. *Photosynthetica* 38, 349-360.

Cybulski W.J., Peterjohn W.T., 1999. Effects of ambient UV-B radiation on the aboveground biomass of seven temperate-zone plant species. *Plant Ecology* 145, 175-181.

Day T.A., Ruhland C.T., Xiong F.S., 2001. Influence of solar ultraviolet-B radiation on Antarctic terrestrial plants: results from a 4-year field study. *Journal of Photochemistry and Photobiology B: Biology* 62, 78-87.

Dorman C.F., Woodin S.J., 1992. Climate Change in the Arctic: using plant functional types in a meta-analysis of field experiments. *Functional Ecology* 16, 4-17.

Flint S.D., Ryel R.J., Caldwell M.M., 2003. Ecosystem UV-B experiments in terrestrial communities: a review of recent findings and methodologies. *Agriculture Forest Metrology* 120, 177-189.

Force L., Critchly C., van Rensen J.J.S., 2003. New fluorescence parameters for monitoring photosynthesis in plants. 1. The effect of illumination on the fluorescence parameters of the JIP-test. *Photosynthesis Research* 78, 17-33.

Gehrke C., Johanson U., Björn L.O., Callaghan T.V., Lee J.A., 1996. Effects of enhanced ultraviolet-B radiation on terrestrial sub-arctic ecosystems and implications for interactions with increased atmospheric CO₂. *Ecological Bulletin* 45, 192-203.

Gwynn-Jones D., Lee A., Callaghan T.V., 1997. Effects of enhanced UV-B radiation and elevated carbon dioxide concentration on sub-arctic forest heath system. *Plant Ecology* 128, 242-249.

Gwynn-Jones D., Johanson U., 1996. Growth and pigment production in the subarctic grass species under four different UV-B irradiation levels. *Physiologia Plantarum* 97, 701-707.

Hansen A.H., Jonasson S.E., Michelsen A., Julkunen-Tiitto R., 2006. Long-term experimental warming, shading and nutrient addition affect the concentration of phenolic compounds in arctic-alpine deciduous and evergreen shrubs *Oecologia* 147, 1-11.

Heredia P., Des Las Rivas J., 2003. Fluorescence induction of Photosystem II membranes shows the steps to reduction and protonation of the quinone pool. *Journal of Plant Physiology* 160, 1497-1504.

Huskies A.H.L., Lud D., Moerdijk-Poortvliet T.J.W., 2001. Field research on the effects of UV-B filters on terrestrial Antarctic vegetation. *Plant Ecology* 154, 77-86.

Klitgaard A.B., Rasch, M. Canning K., 2007. Zackenberg Ecological Research Operations, 12th Annual report 2006 – Copenhagen, Danish Polar Center, Ministry of Science, Technology and Innovation.

Krause G.H., Weis E., 1991. Chlorophyll fluorescence and photosynthesis: The basics. *Annual Review of Plant Physiology and Plant Molecular Biology* 42, 313-349.

Krizek D.T., Mirecki R.M., 2004. Evidence for phytotoxic effects of cellulose acetate in UV exclusion studies. *Environmental and Experimental Botany* 51, 33-43.

Krüger G.H.T., Tsmilli-Michael M., Strasser R.J., 1997. Light stress provokes plastic and elastic modifications in structure and function of Photosystem II in camelia leaves. *Physiologia Plantarum* 101, 265-277.

Madronic S., McKencie R.L., Björn L.O., Caldwell M.M., 1998. Changes in biologically ultraviolet radiation reaching the Earth's surface. *Journal of Photochemistry and Photobiology B. Biology* 46, 5-19.

Meijkamp B.B., Doodeman G., Rozema J., 2001. The response of *Vicia faba* to enhanced UV-B radiation under low and near ambient PAR levels. *Plant Ecology* 154, 137-146.

Paul N., 2001. Plant responses to UV-B: time to look beyond stratospheric ozone depletion? *New Phytologist* 150, 1-8.

Paul N.D., Gwynn-Jones D., 2003. Ecological roles of solar UV radiation: towards an integrated approach. *Trends in Ecology & Evolution* 18, 48-55.

Pontauiller J.Y., 1990. A cheap quantum sensor using a gallium arsenide photodiode. *Functional Ecology* 4, 591-596.

Phoenix G.K., Gwynn-Jones D., Callaghan T.V., Sleep D., Lee J.A., 2001. Effects of global change on a sub-Arctic heath: Effects of enhanced UV-B radiation and increased summer precipitation. *Journal of Ecology* 89, 256-267.

Phoenix G.K., Gwynn-Jones D., Lee J.A., Callaghan T.V., 2003. Ecological importance of ambient solar ultraviolet radiation to a sub-arctic heath community. *Plant Ecology* 165, 263-273.

Rinnan R., Keinänen M.M., Kasurinen A., Asikainen J., Kekki T.K., Holopainen T., Røpoulsen H., Mikkelsen T.N., Michelsen A., 2005. Ambient ultraviolet radiation in the Arctic reduces root biomass and alters microbial community composition but has no effects on microbial biomass. *Global Change Biology* 11, 564-574.

Robson T., Pancotto V.A., Flint S.D., Ballare C.L., Sala O.E., Scopel A.L., Caldwell M.M., 2003. Six years of solar UV-B manipulations affect growth of *Sphagnum* and vascular plants in a Tierra del Fuego peatland. *New Phytologist* 160, 379-389.

Rozema J., Boelen P., Solheim B., Zielke M., Buskens A., Doorenbosch M., Fijn R., Herder J., Callaghan T., Björn L.O., Gwynn-Jones D., Broekman R., Blokker P., van de Poll W., 2006. Stratospheric ozone depletion: High arctic tundra plant growth on svalbard is not affected by enhanced UV-B after 7 years of UV-B supplementation in the field. *Plant Ecology* 182, 121-135.

Ruhland C.T., Day T.A., 2000. Effects of ultraviolet-B radiation on leaf elongation, production and phenylpropanoid concentrations of *Deschampsia antarctica* and *Colobanthus quietensis* in Antarctica. *Physiologia Plantarum* 109, 244-351.

Searles P.S., Flint S.D., Caldwell M.M., 2001. A meta-analysis of plant studies simulating stratospheric ozone depletion. *Oecologia* 127, 1-10.

Shehan J.J., 1996. Sinaopate esters provide greater UV-B attenuation than flavanoids in *Arabidopsis thaliana* (Brassicaceae). *American Journal of Botany* 83, 679-686.

Statistical Analysis Systems Institute (2002) SAS/STAT Users guide. Release 8.2. SAS Institute Inc., Cary, North Carolina, USA

Stirbet A.D., Srivastava A., Strasser R.J., 1998. The energetic connectivity of PSII centers in higher plants probed in vivo by the fast fluorescence rise O-J-I-P and numerical simulations. In: *Photosynthesis: Mechanisms and Effects* (ed Garab G.), pp 4317-4320 Kluwer Academic Publishers, the Netherlands.

Strasser R.J., Srivastava A., Govindjee, 1995. Polyphasic chlorophyll a fluorescence transients in plants and cyanobacteria. *Photochemistry and Photobiology* 61, 32-42.

Strasser B.J., Strasser R.J., 1995. Measuring fast fluorescence transients to address environmental questions: The JIP-test. In: *Photosynthesis: From light to biosphere:*

Proceedings of the Xth international photosynthesis congress (ed Manthis P), pp 977-980, Montpellier-France 1995, Kluwer Academic Publishers, the Netherlands.

Strasser R.J., Tsimilli-Micheal M., Srivastava A., 2000. The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Probing photosynthesis: mechanisms, regulation and adaptation (eds M.Yunus, U. Pathre & P. Mohanty), pp 445-483, Taylor & Francis, London, United Kingdom, ISBN 0-7484-0821-5.

Strasser R.J., Tsimilli-Micheal M., 2001. Stress in plants, from daily rhythm to global changes, detected and quantified by the JIP-Test. *Chimie Nouvelle (SRC)* 75, 3321-3326.

Strasser R.J., Tsimilli-Micheal M., Srivastava A., 2004. Analysis of the chlorophyll a fluorescence transient. In: *Advances in Photosynthesis and Respiration* (eds G.C. Papageorgiou & Govindjee), pp 321-362, Springer, Berlin.

Srivastava A., Guissé B., Greppin H., Strasser R.J., 1997. Regulation of antenna structure and electron transport in Photosystem II of *Pisum sativum* under elevated temperature probed by the fast polyphasic chlorophyll a fluorescence transient: OKJIP. *Biochimica et Biophysica Acta* 1320, 95-106.

Tevini M., Teramura A.H., 1989. UV-B effects on terrestrial plants. *Photochemistry and Photobiology* 50, 479-487.

van Heerden P.D.R., Tsimilli-Michael M., Krüger G.H.J., Strasser R.J., 2003. Dark chilling effects on soybean genotypes during vegetative development: Parallel studies of CO₂ assimilation, chlorophyll a fluorescence kinetics O-J-I-P and nitrogen fixation. *Physiologia Plantarum* 117, 476-491.

Webb A.R., 1997. Monitoring changes in UV-B radiation. In: *Plants and UV-B: Responses to environmental change* (ed Lumsden P.J.), pp 13-30, Cambridge University Press, Cambridge United Kingdom, ISBN 0-521-57222-3.

Weathershead B., Tanskanen A., Stevermer A., 2005. Ozone and Ultraviolet radiation. In: *ACIA, Arctic Climate Impact Assessment* (eds Symon C., Arris L. & Heal B.), pp 151-183, Cambridge University Press, New York, USA.

WMO, 2003. Scientific Assessment of Ozone Depletion: 2002. Global Ozone Research and Monitoring Project. Report no. 47, World Metrological Organization, Geneva.

Xiong F.S., Day T.H., 2001. Effect of solar ultraviolet-B radiation during springtime ozone depletion on photosynthesis and biomass production of Antarctic vascular plants. *Plant Physiology* 125, 738-751.

TABLES:

Table 1:

UV-B radiation recorded by the erythema-weighted sensor at the meteorological station at Zackenberg 2000-2007. Values are in A: Monthly accumulated (MED/month) and B: Monthly maximum (MED/hour).

Table 2:

Concentration of UV-B absorbing compounds for *Salix arctica* and *Vaccinium uliginosum* are given as means \pm 1 standard error. The treatments are: open control (C), transparent filter control (F) UV-B reduction (UV-B) and UV-AB reduction (UV-AB). Treatment differences were tested across sites with 3-way ANOVA in the GLM procedure ($n = 48$ and $\alpha = 0.05$) followed by Tukey's test. The values of F-test and probability of correct null hypothesis are given.

FIGURES

Figure 1:

Fluorescence parameters for *Vaccinium uliginosum*. The treatments are: open control (C), transparent filter control (F) UV-B reduction (UV-B) and UV-AB reduction (UV-AB). The numbers are July means \pm 1 standard error tested with 3-way ANOVA in the GLM procedure ($n = 11355$ and $\alpha = 0.05$). In case of significant treatment effects this was followed by Tukey's test and the treatment differences are indicated with letters.

Figure 2:

Fluorescence parameters for *Salix arctica*. The treatments are: open control (C), transparent filter control (F) UV-B reduction (UV-B) and UV-AB reduction (UV-AB). The numbers are July means \pm 1 standard error tested with 3-way repeated measurements ANOVA in the GLM procedure ($n = 12447$ and $\alpha = 0.05$). In case of significant treatment effects this was followed by Tukey's test and the treatment differences are indicated with letters.

Figure 3:

Spider-plot displaying selected fluorescence parameters for *Salix* (upper) and *Vaccinium* (lower). All values are means across all years and expressed as indexes (relative to that of the filter (F) treatment set to 100%). The treatments are: open control (C), transparent filter control (F) UV-B reduction (UV-B) and UV-AB reduction (UV-AB). The parameters are the initial fluorescence (F_o), maximal fluorescence (F_m), variable

fluorescence (F_v), maximum quantum yield (F_v/F_m), performance index (PI_{Abs}), performance index per leaf cross section (PI_{CS_m}), energy fluxes per leaf cross section (estimated from F_m) of trapping (TR_o/CS_m), dissipation (DI_o/CS_m) and electron transport (ET_o/CS_m) and estimated number of active PSII RC's (RC/CS_m).

Table 1:

A	May	June	July	August
2000	14.21	15.91	13.03	na
2001	na	na	na	7.02
2002	14.71	17.95	12.72	7.00
2003	13.70	17.20	12.50	9.15
2004	15.62	18.12	15.76	8.63
2005	13.35	15.20	13.34	9.95
2006	19.07	24.52	20.81	10.56
2007	18.08	22.31	17.88	7.97

B	May	June	July	August
2000	0.071	0.077	0.072	na
2001	na	na	0.040	0.053
2002	0.077	0.089	0.077	0.053
2003	0.074	0.087	0.070	0.055
2004	0.084	0.089	0.088	0.060
2005	0.070	0.082	0.073	0.069
2006	0.092	0.109	0.098	0.081
2007	0.096	0.103	0.094	0.054

Table 2:

		C	F	UV-B	UV-AB	Fvalue	ProbF
<i>Salix</i>	2001	102.6 ± 3.2 ^A	95.4 ± 4.9 ^{AB}	92.5 ± 4.2 ^{AB}	80.7 ± 3.1 ^B	3.07	0.0365
	2002	97.1 ± 6.6 ^A	87.4 ± 5.4 ^A	85.7 ± 3.5 ^A	81.0 ± 7.9 ^A	1.61	0.2000
	2003	104.5 ± 2.4 ^A	92.7 ± 6.8 ^B	91.5 ± 4.7 ^B	79.7 ± 5.6 ^C	12.01	0.0001
	2005	79.3 ± 9.3 ^A	55.8 ± 7.3 ^A	51.5 ± 5.1 ^A	53.9 ± 5.7 ^A	1.52	0.2603
	2006	106.6 ± 2.9 ^A	98.4 ± 5.4 ^B	82.5 ± 1.7 ^C	83.7 ± 2.3 ^C	15.01	0.0001
<i>Vaccinium</i>	2001	124.8 ± 8.8 ^A	118.5 ± 7.5 ^A	116.2 ± 7.1 ^A	110.8 ± 8.3 ^A	1.36	0.2674
	2002	120.0 ± 5.3 ^A	119.9 ± 4.1 ^A	102.0 ± 3.6 ^B	95.4 ± 3.2 ^C	7.00	0.0005
	2003	113.9 ± 5.2 ^A	108.4 ± 6.0 ^A	107.9 ± 4.8 ^A	97.5 ± 8.6 ^A	2.17	0.1038
	2005	113.9 ± 6.8 ^A	113.9 ± 7.4 ^A	109.2 ± 6.6 ^A	104.0 ± 6.0 ^A	0.47	0.7119
	2006	118.0 ± 4.2 ^A	118.9 ± 4.5 ^A	98.2 ± 5.6 ^B	94.4 ± 5.9 ^B	13.31	0.0001
	2007	102.6 ± 2.2 ^A	91.4 ± 4.1 ^B	81.0 ± 3.3 ^C	80.7 ± 3.9 ^C	11.22	0.0001

Figure 1 *Vaccinium uliginosum*

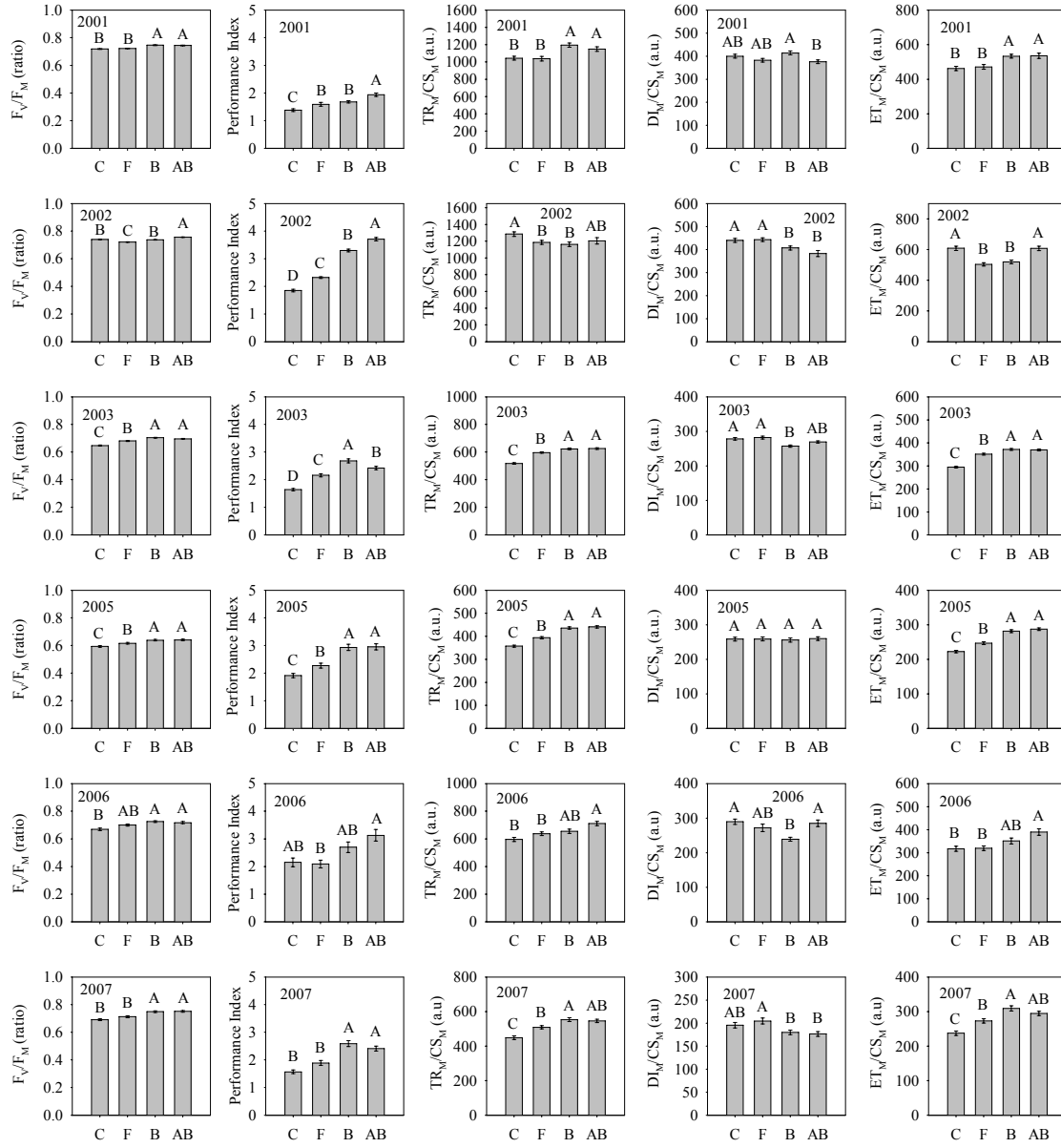


Figure 2 *Salix arctica*

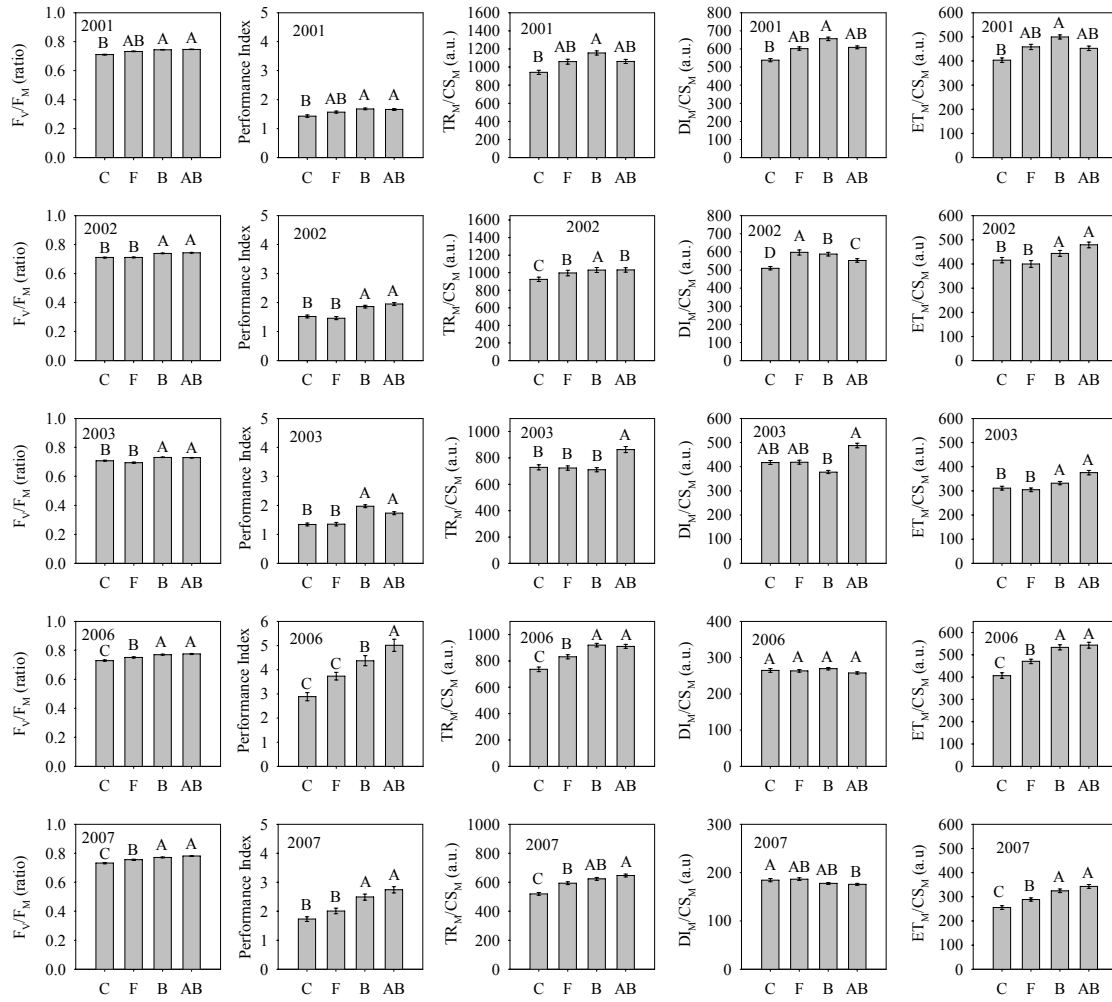
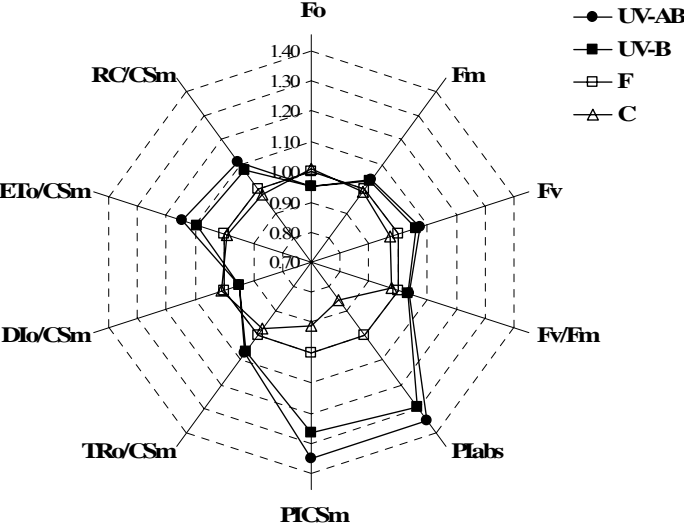
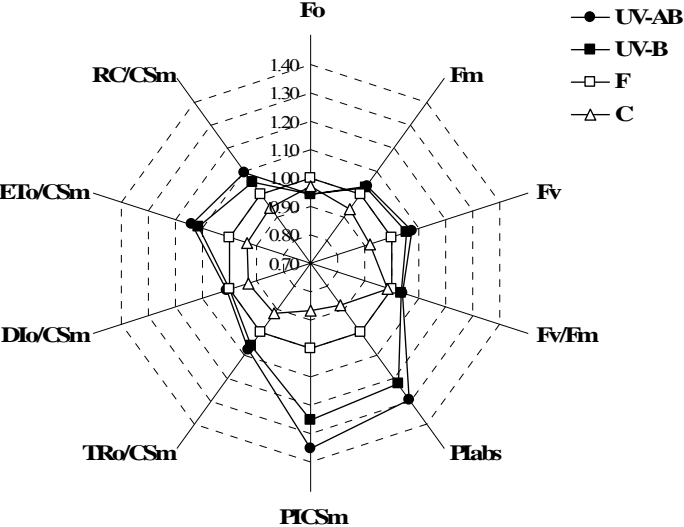


Figure 3:



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